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L2 3431 L1 AND PARATHYROID HORMONE

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LS ANSWER 1 OF 1 MEDLINE
89064600 Document Number: 89064600. PubMed ID: 3197642. Isolation of
16,000-dalton **parathyroid hormone**-like proteins from
two animal tumors causing humoral hypercalcemia of malignancy. Weir E C;
Burtis W J; Morris C A; Brady T G; Insogna K L. (Section of Comparative
Medicine, Yale University School of Medicine, New Haven, Connecticut
06520-5041. J ENDOCRINOLOGY, (1988 Dec) 113 (4) 2744-51. Journal code:
0022-0722. ISSN: 0022-0722. Pub. country: United States. Language: English.

AB A 16K PTH like protein with a unique primary structure has
recently been isolated from several human tumors associated with the
syndrome of humoral hypercalcemia of malignancy. Certain spontaneous and
transplantable animal tumors also cause this syndrome. The responsible
mediator in these animal tumors is not known. We report the isolation of
16K protein from the rat H500 Leydig cell tumor and the canine apocrine
cell adenocarcinoma of the anal sac. Both proteins are potent activators
of PTH receptor-coupled adenylyl cyclase in bone cells. Both
proteins demonstrate similarities in amino acid composition to one another
and to the human PTH-like protein. Limited amino-terminal
sequence information from the canine protein demonstrates homology with

the human **PTH**-like protein. **Antibodies** raised to a synthetic human **PTH**-(1-36)-like peptide cross-react with both the rat and canine proteins in an immunoradiometric assay. These data demonstrate that by physical and immunological criteria **PTH**-like peptides are present in these animal tumors that appear to be closely related to the human **PTH**-like peptide. These data further suggest that this protein is not unique to **humans**, but has an evolutionary origin which extends back at least 65-80 million yr.

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L6 101 DUP REMOVE L4 (36 DUPLICATES REMOVED)

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L7 12 16 AND MONOCLONAL

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PROCESSING COMPLETED FGF L7

L8 22 DUP REMOVE L7 (0 DUPLICATES REMOVED)

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L8 ANSWER 1 OF 22 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2001:256905 Document #: PREV200100256905. Expression of a P2X7 receptor by a subpopulation of human osteoblasts. Gartland, A. (1); Hipskind, R. A.; Gallagher, J. A.; Fowler, W. B.. (1) Human Anatomy and Cell Biology Group, Department of Human Anatomy and Cell Biology, The University of Liverpool, Ashton Street, Liverpool, L69 3GE UK. Journal of Bone and Mineral Research, (May, 2001) Vol. 16, No. 5, pp. 846-856. print. ISSN: 0884-0431. Language: English. Summary Language: English.

AB There is now conclusive evidence that extracellular nucleotides acting via cell surface P2 receptors are important local modulators of bone cell function. Multiple subtypes of P2 receptors have been localized to bone, where their activation modulates multiple processes including osteoblast proliferation, osteoblast-mediated bone formation, and osteoclast formation and resorptive capacity. Locally released nucleotides also have been shown to sensitize surrounding cells to the action of systemic factors such as **parathyroid hormone (PTH)**. In nonskeletal tissue recent attention has focused on one particular P2 receptor, the P2X7 receptor (previously termed P2X), and its ability to form nonselective aqueous pores in the plasma membrane on prolonged stimulation. Expression of this receptor originally was thought to be restricted to cells of hemopoietic origin, in which it has been implicated in cell fusion, apoptosis, and release of proinflammatory cytokines. However, recent reports have indicated expression of this receptor in cells of stromal origin. In this study, we investigated the expression of the P2X7 receptor in two human osteosarcoma cell lines, as well as several populations of primary human bone-derived cells (HBCs) at the levels of messenger RNA (mRNA) and protein. We found that there is a subpopulation of osteoblasts that express the P2X7 receptor and that these receptors are functional as assessed by monitoring ethidium bromide uptake following pore formation. Inhibition of delayed lactate dehydrogenase (LDH) release in response to the specific agonist 2',3'-4-benzoyl-benzyl adenosine triphosphate (BzATP) by the nonspecific P2X receptor antagonist PPADS confirmed a receptor-mediated event. After treatment with BzATP, SaOS-2 cells exhibited dramatic morphological changes consistent with those observed after P2X7-mediated apoptosis in hemopoietic cells. Dual staining with terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end labeling (TUNEL) and a P2X7-specific **monoclonal antibody** confirmed the induction of apoptosis in osteoblasts expressing the P2X7 receptor. These data show for the first time the expression of functional P2X7 receptors in a subpopulation of

osteoblasts, activation of which can result in ATP-mediated apoptosis.

L8 ANSWER 2 OF 22 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2001:112671 Document No.: PREV200100113671. Human pancreatic adenocarcinomas express **parathyroid hormone**-related protein. Bouvet, Michael (1); Nardin, Stephanie P.; Burton, Douglas W.; Behling, Cynthia; Carethers, John M.; Moossa, A. R.; Deftos, Leonard J.. (1) Department of Surgery (112-E), University of California, Veterans Affairs Medical Center, 3350 La Jolla Village Drive, San Diego, CA, 92161: mbouvet@ucsd.edu USA. *Journal of Clinical Endocrinology & Metabolism*, (January, 2001) Vol. 86, No. 1, pp. 310-316. print. ISSN: 0021-972X.
Language: English. Summary Language: English.

AB **PTH**-related protein (PTHrP) is expressed in many common malignancies such as breast and prostate cancer and can regulate their growth. Little is known, however, about the role of PTHrP in pancreatic adenocarcinoma. To study PTHrP in pancreatic exocrine cancer, we studied its expression in pancreatic cancer cell lines and surgical specimens. Eight human pancreatic adenocarcinoma cell lines were evaluated: AsPC-1, BxPC-3, Capan-1, CFPAC-1, MIA PaCa-2, PANC-1, PANC-28, and PANC-48. Murine **monoclonal antibodies** to the amino-terminal (1-34), mid-region (38-64), and carboxyl-terminal peptides (109-141) of PTHrP were used to identify cellular PTHrP and secreted PTHrP, including Western blotting and immunocytochemical staining for PTHrP from each cell line. Cellular PTHrP was detected in all cell line extracts by both Western blotting and immunassay. CFPAC-1, derived from a pancreatic liver metastasis, had the highest concentration of PTHrP, and MIA PaCa-2, derived from primary pancreatic adenocarcinoma, had the lowest. PTHrP was localized by immunocytochemical staining in the cytoplasm in all but one cell line, and both nuclear and cytoplasmic immunostaining were observed in the MIA PaCa-2 and PANC-1 cells. Secretion of PTHrP into cell medium was also observed for each cell line and paralleled intracellular PTHrP levels. Evidence for differential processing of PTHrP expression was provided by studies demonstrating different patterns of PTHrP among the cell lines when assessed by PTHrP immunoassays directed against different PTHrP peptides. In specific, PTHrP secretion measured by a PTHrP-(38-64) assay was highest for BxPC-3, whereas the highest levels of secreted PTHrP-(109-141) occurred in CFPAC-1 and PANC-1. Growth of AsPC-1 cells was stimulated in a dose-dependent manner by PTHrP-(1-34). Immunostaining from archival tissue of patients with pancreatic adenocarcinoma revealed strong PTHrP expression in all 14 specimens. All patients were eucalcemic preoperatively. These results demonstrate that PTHrP is commonly expressed in pancreatic cancer. Our data suggest that PTHrP may have growth-regulating properties in pancreatic adenocarcinoma cells, but further studies are required.

L8 ANSWER 3 OF 22 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2001:103550 Document No.: PREV_0010010550. Development and production of **monoclonal antibodies** against parathyroid hypertensive factor. Krylyva, S. (1); Sutherland, S.; Labedz, T.; Shan, J.; Benishin, G. (1) C3 Technologies, Edmonton, AB Canada. *Journal of Hypertension*, (2000) Vol. 18, No. Suppl. 4, pp. 1-67. print. Meeting Info.: 18th Scientific Meeting of the International Society of Hypertension Chicago, Illinois, USA August 20-24, 2000 International Society of Hypertension. ISSN: 0263-6323. Language: English. Summary Language: English.

L8 ANSWER 4 OF 22 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1999:296674 Document No.: PREV1999020-674. Expression of TRIM31/cyclin D1, retinoblastoma gene products, and Ki67 in parathyroid hyperplasia caused by chronic renal failure versus primary adenoma. Tominaga, Yoshihiko (1); Tsuzuki, Teyonori; Uchida, Kazuharu; Hata, Toshihito; Otsuka, Satoshi; Ichimori, Toshihiko; Yamada, Kazuhiko; Numano, Masahiro; Tanaka, Yuji; Takagi, Hiroshi. (1) Department of Transplant Surgery, Nagoya Second Red Cross Hospital, 2-3 Mycken-cho, Showa-ku, Nagoya, 466 Japan. *Kidney*

International, (April, 1999) Vol. 55, No. 4, pp. 1375-1383. ISSN: 0085-2538. Language: English. Summary Language: English.

AB Background. In primary hyperparathyroidism, certain genetic abnormalities responsible for parathyroid tumorigenesis are proposed, and it has been reported that the overexpression of PPAD1/cyclin D1 induced by a DNA rearrangement of the **parathyroid hormone (PTH)** gene is one of the genetic disorders in a number of primary parathyroid adenomas. However, in secondary hyperparathyroidism caused by uremia, the mechanism of **monoclonal** proliferation in nodular parathyroid hyperplasia is not well understood. To elucidate the mechanism, we examined the expression of PPAD1/cyclin D1, retinoblastoma gene products, and Ki67 in primary adenoma and secondary hyperplasia. Methods. In adenomas (N = 15) and associated glands (N = 7) with normal histology obtained from patients with primary hyperparathyroidism and in diffuse (N = 14), multinodular (N = 18), and single nodular (N = 28) glands from patients who underwent parathyroidectomy for renal hyperparathyroidism, the expression of these cell cycle regulators was evaluated by immunohistochemical technique. A labeling index was used to define the proportion of cells with positive nuclear staining by each **antibody**. Results. In 6 out of 15 (40%) primary adenomas, PPAD1/cyclin D1 was overexpressed (a labeling index of more than 500), possibly because of the **PTH** gene rearrangement, but not in secondary hyperplasia, including single nodular glands. Compared with diffuse hyperplasia, nodular hyperplasia showed a significantly higher expression of PPAD1/cyclin D1 ($P < 0.05$), retinoblastoma gene products ($P < 0.05$), and Ki67 ($P < 0.05$). However, no statistically significant correlation between the expression of PPAD1/cyclin D1 and that of Ki67 was observed in both primary adenoma and secondary hyperplasia. Conclusions. These results suggest that in secondary hyperplasia caused by uremia, at least remarkable overexpression of PPAD1/cyclin D1 induced by **PTH** gene rearrangement may be not the major genetic abnormality responsible for tumorigenesis. Heterogeneous genetic changes seem to contribute to **monoclonal** proliferation of parathyroid cells induced by the expression of PPAD1/cyclin D1 or by some other mechanism independent of the amplification of the proto-oncogene.

L8 ANSWER 5 OF 12 MEDLINE

1998036747 Document Number: 98036747. PubMed ID: 9362424.

Parathyroid hormone-related protein and bone metastases.

Guise T A. (Department of Medicine, University of Texas Health Science Center at San Antonio, 78284-7877, USA.) CANCER, (1997 Oct 15) 80 (8 Suppl) 1572-80. Ref: 67. Journal code: 0361-6320. ISSN: 0361-6320. Pub. country: United States. Language: English.

AB **Parathyroid hormone**-related protein (**PTH**-rP)

was purified and cloned 10 years ago as a factor responsible for the hypercalcemia associated with malignancy. Clinical evidence supports another important role for **PTH**-rP in malignancy as a mediator of the bone destruction associated with osteolytic metastasis. Patients with **PTH**-rP positive breast carcinoma are more likely to develop bone metastasis. In addition, breast carcinoma metastatic to bone expresses **PTH**-rP in 50% of cases, compared with only 1% of metastasis to nonbone sites. These observations suggest that **PTH**-rP expression by breast carcinoma cells may provide a selective growth advantage in bone due to its ability to stimulate osteoclastic bone resorption. Furthermore, growth factors such as transforming growth factor-beta (TGF-beta), which are abundant in bone matrix, are released and activated by osteoclastic bone resorption and may enhance **PTH**-rP expression and tumor cell growth. To investigate the role of **PTH**-rP in the pathophysiology of breast carcinoma metastasis to bone, the human breast carcinoma cell line MDA-MB-231 was studied in a murine model of human breast carcinoma metastasis to bone. A series of experiments were performed in which 1) **PTH**-rP secretion was altered, 2) the effects of **PTH**-rP were neutralized, or 3) the responsiveness to TGF-beta was abolished in

MDA-MB-231 cells. Cultured MDA-MB-231 cells secreted low amounts of **PTH-rP** that increased fivefold in response to TGF-beta. Tumor cells inoculated into the left cardiac ventricle of nude mice caused osteolytic metastasis similar to that observed in **humans** with breast carcinoma. When **PTH-rP** was overexpressed in the tumor cells, bone metastases were increased. MDA-MB-231 cells transfected with the cDNA for human preproPTH-rP secreted a tenfold greater amount of **PTH-rP** and caused significantly greater bone metastases when inoculated into the left cardiac ventricle of female nude mice compared with parental cells. In contrast, when the biologic effects of **PTH-rP** were neutralized or its production was suppressed, such metastases were decreased. Treatment of mice with a neutralizing **monoclonal antibody** to human **PTH-rP** resulted in a decrease in the development and progression of bone metastasis due to the parental MDA-MB-231 cells. Similar results were observed when mice were treated with diazepamethasone, a potent glucocorticoid that suppresses production of **PTH-rP** by the MDA-MB-231 cells *in vitro*. The role of bone-derived TGF-beta in the development and progression of bone metastasis was studied by transfecting MDA-MB-231 cells with a cDNA encoding a TGF-beta type II receptor lacking a cytoplasmic domain, which acts as a dominant negative to block the cellular response to TGF-beta. Stable clones expressing this mutant receptor (MDA/TbetaRIIdeltacyt) did not increase **PTH-rP** secretion in response to TGF-beta stimulation compared with controls of untransfected MDA-MB-231 or those transfected with the empty vector. Mice inoculated into the left cardiac ventricle with MDA/TbetaRIIdeltacyt had fewer and smaller bone metastases as assessed radiographically and histomorphometrically compared with controls. Taken together, these data suggest that **PTH-rP** expression by breast carcinoma cells enhance the development and progression of breast carcinoma metastasis to bone. Furthermore, TGF-beta responsiveness of breast carcinoma cells may be important for the expression of **PTH-rP** in bone and the development of osteolytic bone metastasis *in vivo*. These interactions define a critical feedback loop between breast carcinoma cells and the bone microenvironment that may be responsible for the alacrity with which breast carcinoma grows in bone.

L8 ANSWER 6 OF 22 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1997:289281 Document No.: PREV199793560971. Synovial fluids from patients with osteoarthritis and rheumatoid arthritis contain high levels of **parathyroid hormone**-related peptide. Kohno, Hiraaki; Shigeno, Chihesi (1); Kasai, Ryuichi; Akiyama, Haruhiko; Iida, Hirokazu; Tsuboyama, Tadao; Saito, Kanji; Konishi, Jinji; Nakamura, Takashi. (1) Calcium Lab., Dep. Nuclear Med. Diagn. Imaging, Grad. Sch. Med., Kyoto Univ., Sakyu, Kyoto 606-01 Japan. Journal of Bone and Mineral Research, (1997) Vol. 12, No. 5, pp. 847-854. ISSN: 0884-0431. Language: English.

AB High levels of immunoreactive and biologically active **parathyroid hormone**-related peptide (PTHrP) were detected in synovial fluids from patients with osteoarthritis (OA) and rheumatoid arthritis (RA). The levels of PTHrP immunoreactivity in synovial fluids, measured by a two-site immunoradiometric assay (IRMA) which detects hPTHrP(1-72) or longer peptides and a radioimmunoassay (RIA) specific to the carboxy-terminal portion of hPTHrP, were 3.2 ± 0.3 pmol of hPTHrP(1-72)/l and 61 ± 7.0 pmol of hPTHrP(109-141)/l in OA patients (mean \pm SE, n = 24), and 4.6 ± 0.4 pmol of hPTHrP(1-72)/l and 164 ± 30 pmol of hPTHrP(109-141)/l in RA patients (n = 26). Synovial fluid PTHrP levels distributed above the normal plasma reference ranges in each assay (0.7 - 3.6 pmol of hPTHrP(1-72)/l; i.e. 61.6 pmol of hPTHrP(109-141)/l). After concentration using sequential cation-exchange and reverse-phase chromatography, synovial fluid exhibited the activity that stimulated cyclic adenosine monophosphate (cAMP) accumulation in osteoblastic ROS 17/2.8 cells expressing **PTH/PTHrP** receptors. The cAMP accumulation activity in synovial fluid was sensitive to coincubation with excess hPTHrP(3-40), a **PTH/PTHrP** receptor antagonist, and was

completely neutralized by preincubation with a **monoclonal antibody** specific to hPTHrP but not **PTH**.

Immunohistochemical analysis of RA synovium revealed that PTHrP was localized in fibroblast-like cells in the synovial pannus invading articular cartilage. Our data show that PTHrP is produced locally by the diseased synovial tissue and released into synovial fluid at high concentrations, allowing us to hypothesize that PTHrP plays a novel role as a paracrine/autocrine factor in the pathology of OA and PA.

L8 ANSWER 7 OF 22 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1997:072258 Document No.: PFEV199799563976. Expression and function of a CD4-like molecule in parathyroid tissue. Hellman, Per (1); Karlsson-Parra, Alex; Klareskog, Lars; Rudefelt, Peter; Bjerneroth, Gunnar; Rastad, Jonas; Aherstrom, Goran; Juhlin, Claes. (1) Dep. Surg., Univ. Hosp., S-751 85 Uppsala, Sweden. Surgery (St. Louis), (1996) Vol. 120, No. 6, pp. 925-929. ISSN: 0039-6060. Language: English.

AB Background. Parathyroid tissue expresses the T-lymphocyte antigens CD3 and CD4, and parathyroid CD3 has earlier been proposed to interact in the regulation of parathyroid (**PTH**) release. Methods. Anti-Leu3a, a **monoclonal antibody** recognizing CD4, was used to stain parathyroid tissue immunohistochemically, to influence **PTH** secretion from enzymatically dispersed parathyroid cells, and to immunoprecipitate parathyroid CD4. Northern blot and polymerase chain reaction were used to clarify the similarity between parathyroid and lymphocytic CD4. Serum **PTH** level was measured with an immunoradiometric assay in healthy control subjects and individuals with human immunodeficiency virus type 1. Results. The parenchyma of normal and abnormal parathyroid tissue displayed strikingly variable CD4 expression. Immunoprecipitation showed a 57 kDa molecule, and Northern blot and polymerase chain reaction confirmed the similarity with lymphocyte CD4. Anti-Leu3a inhibited preferentially low calcium-stimulated secretion of **PTH** from dispersed parathyroid cells, without discernible influences on the cytoplasmic calcium concentration of these cells. Individuals with human immunodeficiency virus type 1 displayed significantly lower serum **PTH** levels than healthy control subjects. Conclusions. The results suggest that the human parathyroid chief cell expresses a CD4 moiety, which seems to interact in the **PTH** release in vitro and in vivo and which seems to use another second messenger system than the structurally similar T-cell equivalent.

L8 ANSWER 8 OF 22 BIOSIS COPYRIGHT 2001 BIOLOGICAL ABSTRACTS INC.
1996:193113 Document No.: PFEV199698715241. Immunochemiluminometric assay with two **monoclonal antibodies** against the N-terminal sequence of human **parathyroid hormone**. Gao, Ping; Schmidt-Gayk, Heinrich (1); Dittrich, Karin; Nolting, Bjorn; Maier, Andreas; Roth, Heinz Juergen; Seemann, Oliver; Reichel, Helmut; Ritz, Eberhard; Schilling, Tobias. (1) Endocrine Lab., Lab. Group, Im Breitspiel 15, D-69116 Heidelberg Germany. Clinica Chimica Acta, (1996) Vol. 245, No. 1, pp. 39-59. ISSN: 0009-8981. Language: English.

AB We have developed an immuno-chemiluminometric assay (ICMA) with two **monoclonal antibodies** for the N-terminal sequence of human **parathyroid hormone** (hPTH). One **monoclonal antibody** (A1-79) was physically adsorbed onto polystyrene beads, the other (B1-70) was labelled with acridinium ester and synthetic hPTH (1-84) was used as standard. This assay has cross-reactions with synthetic hPTH (1-34 and 1-84) but no cross-reactions with hPTH (1-18), (18-43), (32-84), (44-86), (53-84) and rPTH-rP (1-86). The assay detection limit is 0.4 pmol/l. The normal range is 1.3-12 pmol/l based on 71 normal volunteers. About 91% of study patients (n = 56) with surgically proven primary hyperparathyroidism (1 degree PHPT) had **PTH** values above normal and one of them showed a low normal intact **PTH** value but elevated **PTH** values with use of this assay. After immunabsorption of plasma samples from

patients with secondary hyperparathyroidism (2 degree HPT) on hemodialysis with polystyrene beads containing **antibodies** against hPTH (39-84), some patients still showed significant amounts of **PTH** in this new ICMA but not intact **PTH**. The data reveal that significant amounts of amino-terminal immunoreactive **PTH** fragments rarely exist in 1 degree HPT but are present in some patients with 2 degree HPT. The major advantage of this assay is to measure both amino-terminal **PTH** fragments and intact **PTH** with no interference from carboxy-terminal **PTH** fragments because two anti-N-terminal hormone sequence **monoclonal antibodies** are used.

L8 ANSWER 9 OF 22 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1995:599229 Document No.: PREV19950514178. Agonist-Stimulated Phosphorylation of the G Protein-Coupled Receptor for **Parathyroid Hormone (PTH)** and **PTH**-Related Protein. Blits, Eberhard; Bamding, Tom; Nissensohn, Robert A. (1) Endocrine Unit, Veterans Affairs Med. Cent., 4150 Clement St., San Francisco, CA 94121 USA. Endocrinology, (1995) Vol. 136, No. 10, pp. 4271-4277. ISSN: 0013-7227. Language: English.

AB The objectives of the present study were to determine whether the G protein-coupled receptor for **PTH** and **PTH**-related protein (PTHRP) is subject to agonist-specific phosphorylation and to characterize the relevant kinase(s). The opossum kidney **PTH** PTHrP receptor stably expressed in human embryonic kidney 293 cells was coupled to adenylyl cyclase, with half-maximal activation occurring in the presence of 0.1 nM bovine (b) **PTH**-(1-34). Immunoprecipitation of extracts of ³²P-labeled cells using a **monoclonal antibody** to the **PTH**/PTHRP receptor revealed the presence of a major ³²P-labeled protein of approximately 85 kilodaltons that was not evident in untransfected 293 cells. bPTH-(1-34) treatment produced a rapid dose-dependent increase in phosphorylation of the 85-kilodalton receptor, with a maximal effect that was 3.5 +/- 0.7-fold (n = 4) over basal. Half-maximal phosphorylation occurred with 10 nM bPTH-(1-34), similar to the hormone concentration required for 50% receptor occupancy. Activation of protein kinase A or protein kinase C with forskolin or phorbol 12-myristate 13-acetate also increased **PTH**/PTHRP receptor phosphorylation, but to a lesser degree than **PTH**. Neither of these kinases mediated the effect of **PTH**, as blockade of the protein kinase A pathway (with H-89) or the protein kinase C pathway (with the bisindolylmaleimide GF 109203X) did not inhibit bPTH-(1-34)-induced **PTH**/PTHRP receptor phosphorylation. These results suggest that agonist stimulated **PTH**/PTHRP receptor phosphorylation may involve a nonsecond messenger-activated kinase, such as a member of the G protein-coupled receptor kinase family.

L8 ANSWER 10 OF 22 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1996:57536 Document No.: PREV19960809071. Expression and function of a CD3-like molecule on normal and abnormal human parathyroid cells. Hellman, Per (1); Uthlin, Claes; Karlsson-Parras, Alex; Klareskog, Lars; Rudefelt, Peter; Rastad, Jonas; Akerblom, Goran. (1) Dep. Surg., Univ. Hosp., S-751 17 Uppsala, Sweden. Surgery (St Louis), (1995) Vol. 117, No. 4, pp. 1305-1302. ISSN: 0039-6160. Language: English.

AB Background. Normal and abnormal human parathyroid tissue express the T-lymphocyte protein CD4, and parathyroid and lymphocyte cells show similarities with respect to mechanisms of calcium permeability and regulation of the cytosolic calcium concentration. Method. Anti Leu4, a **monoclonal antibody** recognizing the T-lymphocyte glycoprotein complex CD3, is used to immunohistochemically stain normal and abnormal human parathyroid cells and to explore influences on the **parathyroid hormone (PTH)** secretion of enzymatically dispersed parathyroid cells. Results. Parathyroid glands of patients with different forms of hyperparathyroidism displayed variable

expression of the anti-CD3 reactive complex. The stainings correlated both positively and inversely to immunoreactivity for a previously defined calcium sensor, the decreased expression of which may constitute a molecular basis for hyperparathyroidism. Incubation of parathyroid cells with the anti-Leu4 **antibody** inhibited **PTH** secretion and reduced its sensitivity to external calcium without influence on parathyroid cytoplasmic calcium concentration. Conclusions. The results suggest that the human parathyroid cells express a CD3-like molecule with the ability to interact in **PTH** release.

L8 ANSWER 11 OF 22 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1995:360066 Document No.: PREV199598374366. A case of acute lymphoblastic leukemia accompanied with the production of **parathyroid hormone**-related protein. Harutsumi, Makoto (1); Akazaki, Ayumi; Kitamura, Tetsuro; Manki, Akira; Tanaka, Hiroyuki; Oda, Mayumi; Seino, Yoshiki. (1) Dep. Pediatrics, National Iwakuni Hosp., 2-5-1 Murasico machi, Iwakuni-shi, Yamaguchi 740 Japan. Mineral and Electrolyte Metabolism, (1995) Vol. 21, No. 1-3, pp. 171-176. ISSN: 0375-0392. Language: English.

AB Hypercalcemia accompanied with malignant tumors is generally classified into two categories, namely with or without bone metastasis. As for the latter, bone resorption-stimulating factors produced by tumor cells, such as **parathyroid hormone**-related protein (PTHrP), show hormone-like effects and promote a bone resorption. Many cases have been reported regarding the production of PTHrP in adult T cell leukemia (ATL), but few have been reported with acute lymphoblastic leukemia (ALL). We report here a similar case with ALL. A 12-year-old male presented with fever, petechiae and thrombocytopenia, and was diagnosed as ALL. We started the induction therapy and confirmed complete remission. Later, he relapsed 3 times without symptoms apart from hypercalcemia at the beginning. Elevation of the serum calcium level followed by a rise of lymphoblastic cells was recognized. Bone metastasis was excluded since bone mineral density and serum mid region **PTH** were normal and no abnormal findings were noticed on X rays and ^{99m}Tc bone scintigraphy. However, his urinary PTHrP level was high, and his lymphoblastic cells staining immunocytochemically with the **monoclonal antibodies** against the C-terminal region of PTHrP showed a positively brownish color. Finally, he died of pulmonary aspergillosis. Hypercalcemia was not related to serum **PTH** or bone metastasis. ATL viral infection reported as a cause of PTHrP production was also excluded from several experimental data. Therefore, we concluded that lymphoblastic cells directly produced PTHrP, and that this PTHrP played an important role in the induction of hypercalcemia.

L8 ANSWER 12 OF 22 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1995:411461 Document No.: PREV199598425761. The tumor markers CEA, CA 15-3, MCA, TFA, ferritin and **PTH** in the diagnosis and monitoring of primary breast cancer. Lemyagin, V. P.; Vysetskaya, I. V.. Oncol. Res. Cent., Russ. Acad. Med. Sci., Moscow Russia. Vestnik Rossiiskoi Akademii Meditsinskikh Nauk, (1995) Vol. 0, No. 4, pp. 10-14. Language: Russian. Summary Language: English.

AB Sera from 154 females were assayed for markers of tumor growth, such as cancerembryonal antigen (CEA), breast cancer-associated antigen (CA 15-3), mucinoid cancer antigen (MMA), ferritin, tissue polypeptide antigen (TPA), parathyroid hormone (**PTH**) by means of **monoclonal antibody** kits by using radioimmunoassay and enzyme immunoassay. Forty nine patients were diagnosed as having benign breast neoplasms, 171 patients were admitted to the clinic for primary breast cancer of varying severities, 34 patients without any breast abnormalities and somatic diseases comprised a control group. There was a close correlation between the higher levels of markers and the stage of a tumorous process. Various therapies-induced decreases in the levels of the markers serve as an objective criterion for the efficiency of the latter. Steady increases in the mean concentrations of tumor markers in the intra- and

posttherapeutic periods are indicative of an extremely poor prognosis in this group of patients.

L8 ANSWER 13 OF 22 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1994:527349 Document No.: PREV199497540349. Development and clinical application of an immunofluorometric assay for intact **parathyroid hormone**. Vieira, J. G. H. (1); Nishida, S. K.; Kasamatsu, T. S.; Amarante, E. C.; Kunii, I. S.. (1) Escola Paulista Med., Caixa Postal 20266, 04034-970 Sao Paulo, Sao Paulo Brazil. Brazilian Journal of Medical and Biological Research, (1994) Vol. 27, No. 10, pp. 2379-2382. ISSN: 0100-879X. Language: English.

AB **Parathyroid hormone (PTH)** is a linear peptide of 84 amino acids that is found in serum mainly in the form of carboxyl terminal fragments. The biological activity of **PTH** depends on the presence of the amino-terminal portion and in circulation is limited to the intact molecule. We describe an immunofluorometric assay for the measurement of **PTH**-(1-84) based on a chicken egg yolk derived amino-terminal **antibody** bound to microtiter plates by an anti-chicken Ig **monoclonal antibody**. As tracer **antibody** we employed a Europium-labelled carboxyl-terminal specific **monoclonal antibody** produced from a mouse immunized with hPTH-(53-84)-BSA conjugate. The assay included an initial overnight incubation of the sample and the solid phase-bound amino-terminal **antibody**, followed by washing and addition of the tracer **antibody**, and an additional two hours of incubation prior to fluorescence reading. The least-detectable dose was in the order of 2.5 pg/ml and preliminary studies in 40 normal adults showed values in the range of 4 to 70 pg/ml; for 12 patients with surgery-proven primary hyperparathyroidism values ranged from 10 to 743 pg/ml and for 34 patients with humoral hypercalcemia of malignancy from 2.5 to 66 pg/ml. We conclude that this assay, with its increased sensitivity and specificity, will be a valuable tool in the study of **PTH** secretion in normal and pathological situations.

L8 ANSWER 14 OF 22 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1995:33222 Document No.: PREV199598047522. Development of a sensitive two-site immunoradiometric assay for **parathyroid hormone**-related peptide: Evidence for elevated levels in plasma from patients with adult T-cell leukemia/lymphoma and B-cell lymphoma. Ikeda, Kyoji (1); Ohno, Hideto; Hane, Motomu; Yokoi, Hiroyuki; Okada, Masato; Honna, Tamotsu; Yamada, Akiko; Tatsumi, Yoshiko; Tanaka, Tsuyoshi; Saitoh, Takao; Hirase, Fumio; Miri, Seiko; Takeuchi, Yasuhiro; Fukumoto, Seiji; Terukina, Shigeharu; Iguchi, Haruo; Kuriyama, Takeshi; Ogata, Etsuro; Matsunaga, Toshio. (1) Div. Endocrinol., Fourth Dep. Internal Med., Univ. Tokyo Sch. Med., 3-28-6 Mejirodai, Bunkyo, Tokyo 113 Japan. Journal of Clinical Endocrinology & Metabolism, (1994) Vol. 79, No. 5, pp. 1322-1327. ISSN: 0021-972X. Language: English.

AB We have developed a sensitive immunoradiometric assay for **PTH**-related peptide (PThrP) using a **monoclonal antibody** against PTHrP(1-34) and a polyclonal **antibody** against PTHrP(55-87), with recombinant human PTHrP(1-87) as the standard. The detection limit of the immunoradiometric assay was 1.5 pmol/L, and plasma PTHrP(1-87) concentrations in 113 healthy subjects were 0.84 ± 0.01 pmol/L, with the upper limit of the normal range being 1.1 pmol/L. Increased circulating PTHrP(1-87) concentrations were demonstrated in all 46 cancer patients with hypercalcemia, but not in patients with primary hyperparathyroidism, chronic renal failure, or hypoparathyroidism. Normalization of serum calcium levels after resection of tumors was shown to correlate well with that of plasma PTHrP(1-87) concentrations in 22 cancer patients. High circulating PTHrP(1-87) levels were also demonstrated in 12 out of 13 hypercalcemic patients with adult T-cell leukemia/lymphoma and in 7 out of 9 hypercalcemic patients with non-Hodgkin's lymphoma especially of B-cell type. These results suggest

that PTHrP is a major humoral factor responsible for the hypercalcemia frequently associated with adult T-cell leukemia/lymphoma and also with B-cell lymphoma.

L8 ANSWER 15 OF 22 MEDLINE

94283265 Document Number: 94283265. PubMed ID: 8013360.

Parathyroid hormone-related protein in the cardiovascular system. Burton D W; Brandt D W; Deftos L J. (Department of Medicine, University of California-San Diego 92161.) ENDOCRINOLOGY, (1994 Jul) 135 (1) 253-61. Journal code: 0375040. ISSN: 0013-7227. Pub. country: United States. Language: English.

AB **PTH**-related protein (PTHrP) is expressed in a stretch-responsive manner in several types of smooth muscle. We previously demonstrated the production of PTHrP in adult rat heart muscle. In this study, we demonstrate the production of PTHrP in the cardiovascular systems of several mammalian species, including human. We demonstrate PTHrP by immunohistochemistry, using a panel of murine **monoclonal antibodies** to PTHrP epitopes that span the entire length of the human PTHrP amino acid sequence, quantitate the concentration of PTHrP in the rat cardiovascular system by region-specific RIAs, and measure the relative levels of PTHrP messenger RNA (mRNA) by competitive polymerase chain reaction. Immunohistochemistry studies demonstrated the presence of PTHrP in the cardiovascular systems of **humans**, rats, pigs, and rabbits. The most robust expression was found in atria, followed by the large vessels, then ventricles. No difference was seen between the left and right sides of the heart. Double staining procedures revealed that PTHrP and atrial natriuretic peptide were coexpressed in some cells. Using RIAs and polymerase chain reaction, we demonstrated that atria contained a higher concentration of PTHrP than ventricles and that the relative PTHrP concentrations correlated to its mRNA concentrations in these two tissues. The concentrations of PTHrP in the smooth muscle surrounding the aorta and vena cava were comparable to those in atria. However, in these large vessels, the higher PTHrP levels did not correspond to its mRNA levels. Whereas the immunoreactive concentrations of PTHrP were similar in the atria, aorta, and vena cava, the mRNA levels in the aorta and vena cava were 3-fold lower than those in the atria. Certain PTHrP epitopes appeared to be differentially expressed in specific cardiovascular tissues. A comparison of region-specific assays showed that immunoreactivity, measured by immunoassays to PTHrP-(33-64) and PTHrP-(109-141) were 3- to 5-fold greater than that determined by an immunoassay to PTHrP-(1-34). Our observations demonstrate that the atria, aorta, and vena cava contain the greatest amounts of PTHrP in the cardiovascular system. The discrepancy between the concentrations of PTHrP and its mRNA present in the aorta and vena cava suggest that the two may be regulated differently in these tissues. The widespread distribution of PTHrP suggests an important function for the protein in the cardiovascular system, possibly functioning as the calcium counterpart for the atrial natriuretic-sodium regulatory axis.

L8 ANSWER 16 OF 22 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

1994:16397 Document No.: PMSV199427071157. Regulation of proliferation in JEG-3 cells by a 50-kDa Ca-2+ sensor and **parathyroid hormone**-related protein. Hellman, Per (1); Hellman, Bjorn; Juhlin, Olles (1); Jappner, Harald; Rastad, Jonas; Riedel, Peter; Akerstrom, Goran. (1) Dep. Surgery, Univ. Hosp., S-751 35 Uppsala Sweden. Archives of Biochemistry and Biophysiology, (1993) Vol. 207, No. 2, pp. 319-325. ISSN: 0003-9861. Language: English.

AB JEG-3 cells are derived from human trophoblasts and demonstrated to express a 500-kDa Ca-2+ sensing protein, which elicits biphasic elevations of cytoplasmic Ca-2+ concentrations ([Ca-2+]i) and mediates Ca-2+ regulation of **parathyroid hormone**-related protein (PTHrP) release from placental cytotrophoblasts. Cytocentrifuged JEG-3 cells were immunostained by **monoclonal** and polyclonal antiserum

toward PTHrP(1-34) and (38-64). Elevation of external Ca-2+ from 0.5 to 3.0 mM induced only a sluggish rise in (Ca-2+)i and no stimulation of cAMP production despite a more than twofold elevation of PTHrP(1-34) release.

Monoclonal antibodies recognizing functionally discrepant epitopes of the Ca-2+ sensor protein substantiated uncoupling of this sensor in the Ca-2+-regulated PTHrP release. Exogenous activation of protein kinase C by a phorbol ester strongly augmented the secretion of PTHrP(1-34), whereby uncoupling of the Ca-2+ sensor was partially reversed. This functional differentiation was associated with reduced (3H)thymidine incorporation in JEG-3 cells. Proliferation of these cells was inhibited by 71% upon rise of extracellular Ca-2+ from 0.5 to 3.0 mM, and this inhibition was abolished by **antibody**-mediated interference with the Ca-2+ sensor function. PTHrP(1-36) and **PTH** (1-34) at concentrations up to 10⁻⁷ M decreased proliferation and stimulated the cAMP content of JEG-3 cells. The findings support simultaneous Ca-2+ sensor and **PTH**/PTHrP receptor expression in JEG3 cells, and that Ca-2+ inhibits proliferation by actions on the Ca-2+ sensor as well as by stimulation of PTHrP release possibly mediating autocrine growth inhibition.

L8 ANSWER 17 OF 22 BIOSIS COPYRIGHT 2001 BIOLOGICAL ABSTRACTS INC.
1993:1881:8 Document No.: FREV19930908583. In situ correlation of synthesis and storage of parathormone in parathyroid gland disease. Kendall, C. H. (1); Potter, Linda; Brown, R.; Jasani, B.; Pringle, J. H.; Lauder, I.. (1) Dep. Histopathol., Leicester Royal Infirmary, Leicester LE1 5WW UK. Journal of Pathology, (1993) Vol. 149, No. 1, pp. 61-66. ISSN: 0022-3417. Language: English.

AB The distribution and expression of **parathyroid hormone** (**PTH**) were investigated in normal and abnormal parathyroid tissue. **PTH** was detected using a **monoclonal antibody** with specificity for the 44-68 region of the **PTH** molecule. Prominent reactivity for **PTH** was seen in normal parathyroid with a granular pattern of staining. Active parathyroid tissue (adenoma and hyperplasia) showed much less reactivity for **PTH**, although there was prominent reactivity in the normal tissue around adenomas. Comparison of expression of **PTH** with that of parathormone mRNA showed a reciprocal pattern in normal tissue and, to a less marked extent, in abnormal tissue. Parathyroid carcinoma in particular had coinciding areas of **PTH** and **PTH** mRNA expression. Oxyphil cells had little or no **PTH** expression, except in the associated 'colloid' in some cases. The findings indicate an inverse relationship between storage and cellular synthesis of **PTH**, this being more marked in physiological than in pathological conditions of the parathyroid.

L8 ANSWER 18 OF 22 CAPLUS COPYRIGHT 2001 ACS
1993:1244 Document No. 118:1244 The expression of **parathyroid hormone** related protein (PTHrP) in normal parathyroid: histochemistry and in situ hybridization. Kitazawa, Riko; Kitazawa, Shhei; Fukase, Masaaki; Fujita, Takao; Kobayashi, Akira; Chihara, Kazuo; Matsui, Takanori; Mori, Kuniyuki; Kido, Aki, Japan. Histochemistry, 97(4), 211-19 (English) 1992. CODEN: HEMYAL. ISSN: 0301-5564.

AB The expression and localization of **PTH** hormone-related protein (PTHrP), a major factor responsible for humoral hypercalcemia of malignancy (HHM), was investigated in 14 cases of surgically resected normal parathyroid gland. For light microscopy immunohistochem., formalin-fixed and paraffin-embedded specimens were stained with avidin-biotin peroxidase complex (ABC) by using the anti-PTHrP **monoclonal antibody** (MoAb), 4B3. Four percent paraformaldehyde (PFA)-fixed and OCT compd.-embedded specimens were used for pre-embedded immunoelectron microscopy. For in situ hybridization, 4% PFA-fixed, frozen sections were studied using a bromodeoxyuridine (BrdU)-labeled PTHrP cDNA probe. Immunohistochem., 12 of the 14 cases

were pos. for PTHrP, which was obsd. mainly in the oxyphil and transitional oxyphil cells. The chief and clear cells, on the other hand, were faintly pos. Electron microscopically, secretory granules pos. for PTHrP were obsd. in cells contg. abundant mitochondria. Consistent with the PTHrP immunoreactivity, transcripts of PTHrP were obsd. also in the oxyphilic cells by *in situ* hybridization. Thus, the prodn. and secretion of PTHrP was shown by the oxyphil cell lineage in the normal parathyroid glands.

L8 ANSWER 19 OF 22 CAPLUS COPYRIGHT 2002 ACS
1991:226819 Document No. 114:226819 Immunological identification and distribution of **parathyroid hormone**-like protein polypeptides in normal and malignant tissues. Kramer, Steven; Reynolds, Frederick H., Jr.; Castilla, Macarena; Valenzuela, David M.; Thorikay, Midori; Scoville, John M. (Chryogene Sci., Inc., Manhasset, NY, 11030, USA). Endocrinology (Baltimore), 132(4), 1427-37 (English) 1991. CODEN: ENDOAG. ISSN: 0013-7227.

AB Monoclonal and polyclonal **antibodies** recognizing human **parathyroid hormone**-like protein (PTHLP) have been produced using a series of recombinant and synthetic PTHLP peptides. These **antibodies** have been used to develop a two-site immunometric enzyme immunoassay which detects PTHLP[1-87] and PTHLP[1-141] but not PTH. The immunoassay detected PTHLP in exts. of squamous carcinomas and normal tissues at concns. from 7-515 ng PTHLP[1-87]/mg protein. Immunoblotting of the ext. which showed the highest immunoreactivity, a squamous carcinoma of the lung from a patient with hypercalcemia, revealed a major band of mol. wt. 26,500 and several other higher mol. wt. bands. Similar polypeptides were obsd. by immunoblotting cell exts. from a cell line, SCoBER, which secretes immunoreactive PTHLP into its medium and also from tumors in nude mice derived from this cell line. Chelating agents did not alter the immunoblotting pattern, and **antibodies** to three different epitopes of PTHLP recognized these bands, indicating PTHLP expression in the exts. Immunohistochem. staining of normal human tissue with these **antibodies** revealed several PTHLP-contg. tissues and confirmed the results of the immunoassay, suggesting a paracrine role for PTHLP. Staining was obsd. in several neoplastic tissues including squamous cell carcinomas, lung carcinoma, bladder carcinoma, osteogenic sarcoma, and adenocarcinoma of the colon.

L8 ANSWER 20 OF 22 CAPLUS COPYRIGHT 2002 ACS
1989:444651 Document No. 111:244651 Pathogenesis of malignancy-associated hypercalcemia; **parathyroid hormone**-related protein (PTH-rP). Satoh, Kanji; Kasano, Keizo; Imamura, Hidehito; Fujii, Yuko (Tokyo Women's Med. Coll., Tokyo, Japan). Pharma Med., 7(4), 25-30 (Japanese) 1989. CODEN: PMEDEC. ISSN: 0289-5803.

AB A review, with 24 refs., discussing the role of **parathyroid hormone**-related protein (PTH-rP) in pathogenesis of malignancy-assocd. hypercalcemia and possible treatment of the disease with **PTH-rP monoclonal antibodies** and **PTH** antagonists.

L8 ANSWER 21 OF 22 CAPLUS COPYRIGHT 2002 ACS
1988:431121 Document No. 119:21121 Hyperparathyroidism is associated with reduced expression of a parathyroid calcium receptor mechanism defined by **monoclonal anti-parathyroid antibodies**. Juhlin, C.; Blaerup, L.; Nygren, P.; Ljunghall, S.; Sylje, E.; Kastad, J.; Aakerstrom, G. (Dep. Surg., Univ. Uppsala, Uppsala, S-751 85, swed.). Endocrinology (Baltimore), 122(6), 2999-3001 (English) 1988. CODEN: ENDOAG. ISSN: 0013-7227.

AB Parathyroid tissue from patients with hyperparathyroidism (HPT) exhibited reduced immunohistochem. reactivity with **monoclonal anti-parathyroid antibodies**, previously shown to stain intensely

the surface of normal human parathyroid cells and to interfere with a receptor mechanism of these cells which is involved in the sensing and gating of Ca²⁺. **Parathyroid hormone (PTH)** release and cytoplasmic Ca²⁺ concns. of dispersed cells from the pathol. parathyroid glands had right-shifted dependencies on extracellular Ca²⁺, and exposure to the **antibodies** rendered both Ca²⁺ and **PTH** release almost completely insensitive to changes in ambient Ca²⁺. Reduced expression of a parathyroid Ca²⁺ receptor mechanism may be an important cause for the aberrant **PTH** release in HPT.

L8 ANSWEF 22 OF 22 CAPLUS COPYRIGHT 2002 ACS
1986:551143 Document No. 105:151143 **Monoclonal antibodies** against **parathyroid hormone**. Nussbaum, Samuel R.; Lin, C. Shirley; Feiner, B.; Vitale, J.; Rosenthal, A. S.; Rosenblatt, M.; Potts, J. T., Jr. Harvard Med. Sch., Massachusetts Gen. Hosp., Boston, MA, 02114, USA. Serono Symp. Publ. Raven Press, 30 (Monoclonal Antibodies), 107-16 (English: 1986). CODEN: SPRPDU. ISSN: 0736-897X.

AB Human **parathyroid hormone** 1-34 [**PTH**-(1-34)] was prep'd. and mice were immunized with this hormone. Using several inbred or congenic mouse strains, the immune response was shown to be controlled by I-A^k and I-E^k genes of the murine H-2 complex. This explains the previously obsd. low response of BALB/c mice (which are H-2d). Based on this genetics knowledge, a higher-responder strain (C3H.Ke) was selected and a protocol for the prepn. of high-affinity **monoclonal antibodies** to **PTH**-(1-34) is described using this strain.

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FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 15:34:23 ON 22 SEP 2002

L1 2348339 S ANTIBODY
L2 3431 S L1 AND PARATHYROID HORMONE
L3 1559 S L2 AND PTH
L4 137 S L3 AND HUMANS
L5 1 S L4 AND DOGS
L6 101 DUP REMOVE L4 (36 DUPLICATES REMOVED)
L7 32 S L6 AND MONOCLONAL
L8 32 DUP REMOVE L7 (0 DUPLICATES REMOVED)

=: s 10 and polyclonal
L9 7 L6 AND POLYCLONAL

=: dup remove 19
PROCESSING COMPLETED FOR L9
L10 7 DUP REMOVE L9 (0 DUPLICATES REMOVED)

=: d 110 1-7 abib abs

L10 ANSWER 1 OF 7 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
2002034953 EMBASE Plasma levels of insulin-like growth factor binding protein 4 (IGFBP-4) under normal and pathological conditions. Van Doorn, J.; Cornelissen, A.J.F.H.; Van Buul-Offers, S.C.; Dr. J. Van Doorn, Department of Paediatrics, Wilhelmina Children's Hospital, University Medical Center Utrecht, PO Box 85000, 3500 AB Utrecht, Netherlands. J.vandoorne@lab.azu.nl. Clinical Endocrinology 54/5 (655-664) 2001. Refs: 40.

ISSN: 0300-0664. CODEN: CLENAQ. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB OBJECTIVE: Insulin-like growth factor binding protein-4 (IGFBP-4) belongs

to a family of six structurally related IGF-binding proteins that are involved in the modulation of the biological effects of the IGFs. In order to obtain more insight into the clinical significance and regulation of IGFBP-4 in vivo we determined the levels of this protein by a specific radioimmunoassay in the human circulation under normal and varicous pathological conditions. DESIGN AND PATIENTS: Selected human biological fluids and plasma samples from 804 normal healthy males and females, ranging from 0 to 78 years of age, were analysed. In addition, plasma samples from patients with several disorders (i.e. hypothyroidism, hyperthyroidism, GH-deficiency, acromegaly, cancer, chronic renal failure corticosteroid-treatment) were investigated. MEASUREMENTS: A specific RIA for hIGFBP-4 was developed, using a rabbit **polyclonal antibody** raised against a synthetic peptide containing amino acids 8-103 of the mature hIGFBP-4 sequence. RESULTS: In normal individuals circulating IGFBP-4 levels in males did not change with age. For females the values tended to increase slightly in older age. Overall, the mean 11.1 ± 1.0 $\mu\text{g/l}$ for males and females (19.9 ± 1.3 $\mu\text{g/l}$ and 19.3 ± 1.7 $\mu\text{g/l}$, respectively) were not different. Narmative range values of IGFBP-4 correlated weakly with those of IGF-II ($r = 0.31$, $P < 0.001$). Neither hypothyroidism nor hyperthyroidism appeared to influence circulating IGFBP-4 levels since the levels were within the normal range. Both GH status and pharmacological doses of glucocorticoids, as employed in various chronic diseases, did not seriously affect plasma IGFBP-4 either. Under conditions with increased circulating **PTH** levels, i.e. dialysed adult patients with chronic renal failure (CRF) and subjects with hyperparathyroidism, a weak positive relationship was noted between the plasma contents of IGFBP-4 and **PTH**. An excess of IGFBP-4 was found in plasma of both nondialysed and dialysed prepubertal growth retarded children with chronic renal failure (CRF) (mean SDS: 10.75 and 11.78, respectively). IGFBP-4 levels were inversely related to glomerular filtration rate. Similar results were obtained for dialysed adults with CRF. In a group of CRF children who had undergone renal transplantation, circulating IGFBP-4 levels were markedly lower (mean SDS: 3.75). There was no evidence for an increased secretion of IGFBP-4 in the circulation of most of the cancer patients with solid tumours. Several children with acute lymphoblastic leukaemia, however, showed elevated plasma IGFBP-4 levels (mean SDS: 1.27). The presence of IGFBP-4 could also be demonstrated in other human biological fluids. The highest amounts were found in amniotic fluid (391-717 $\mu\text{g/l}$) and follicular fluid (249-500 $\mu\text{g/l}$). CONCLUSIONS: Measurement of plasma IGFBP-4 has been shown so far to be of minor clinical relevance. However, the results indicate that different concentration gradients between plasma and varicous other body fluids may exist. Therefore, it may well be that certain pathophysiological stimuli induce significant alterations in the local turnover rate of IGFBP-4 but that they are not reflected by changes in the circulating levels. The possibility of quantifying IGFBP-4 by RIA will facilitate further in vitro and in vivo studies on its regulation and function in **humans**.

L10 ANSWER 2 OF 7 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC
Document No.: PREVIO00182375. Calcium-sensing receptor expression and signalling in human parathyroid adenomas and primary hyperplasia. Corbetta, Sabrina; Mantovani, Giovanna; Lanis, Andrea; Borga, Stefano; Vicentini, Leonardo; Beretta, E.; Faglia, Giovanni; Di Blasio, Anna Maria; Spada, Anna (i). (i) Istituto di Scienze Endocrine, Ospedale Maggiore IRCCS, Via Francesco Sforza 35, 21100, Milano Italy. J Clin Endocrinol, 2001, Vol. 112, No. 3, pp. 339-345. ISSN: 0021-6644. Language: English. Summary Language: English.

AB OBJECTIVE: Both in vivo and in vitro evidence indicates that primary hyperparathyroidism is characterized by a reduced sensitivity to extracellular calcium ($[\text{Ca}^{2+}]_c$). The existence of alterations in the expression and signalling of calcium sensing receptor (CaSR) in parathyroid neoplasia is still uncertain. In order to clarify the role of

CaSR in the reduced $(\text{Ca}^{2+})_o$ sensing of parathyroid neoplasia we investigated **PTH** secretion and intracellular effectors triggered by CaSR activation as well as the levels of expression of CaSR and CaSR coupled G proteins (Gq/G11) in parathyroid adenomas and primary hyperplasia. MATERIALS AND METHODS: The study included 27 parathyroid adenomas, 4 cases of primary hyperplasia and pools of normal parathyroid biopsies. Tissues were either snap frozen in liquid nitrogen or placed in sterile medium for cell dispersion. The effects of increasing $(\text{Ca}^{2+})_o$ on *in vitro* **PTH** release, intracellular cAMP levels and intracellular calcium $([\text{Ca}^{2+}]_i)$ in cells loaded with the Ca^{2+} indicator fura-2 were evaluated. CaSR mRNA levels were assessed by semi-quantitative RT-PCR analysis, using GAPDH as internal standard, while CaSR protein was detected by western blot analysis using a specific **polyclonal antibody**. Purified antisera selective for G₁alpha and G_qalpha were used to detect this class of proteins. RESULTS: In basal conditions (at 0.1 mM $(\text{Ca}^{2+})_o$) *in vitro* **PTH** released ranged from 3.4 to 118 fmol/60 minutes. Increasing $(\text{Ca}^{2+})_o$ from 0.5 to 1, 2.5 and 5 mM caused a variable effect. One group (n=7) showed a significant but partial reduction of **PTH** release (of 17 to 60% of basal levels) that occurred at physiological $(\text{Ca}^{2+})_o$ concentrations (1 mM), while the remainder showed either inhibition detectable only at 2.5 mM (n=15) or total (n=3) resistance to $(\text{Ca}^{2+})_o$. In the responsive cells, $(\text{Ca}^{2+})_o$ (1-5 mM) caused a pertussis toxin-insensitive $(\text{Ca}^{2+})_i$ rise ranging from 10% to 260%, due to Ca^{2+} release from intracellular stores, and an inhibition of forskolin-stimulated cAMP levels. By RT-PCR almost all tumours tested showed a substantial reduction in CaSR mRNA levels when compared to the normal tissue (CaSR/GAPDH ratio: 3.1 +/- 0.5 vs. 15.3 +/- 3.1; P < 0.001), which was confirmed by immunoblotting analysis demonstrating low levels of CaSR protein in tumour tissues. Moreover, low amounts of G₁alpha and G_qalpha, the G proteins involved in CaSR coupling, were observed in the majority of pathological tissues. CONCLUSIONS: The study shows that the activation of the calcium sensing receptors expressed in adenomatous parathyroid glands modulates intracellular effectors in a similar way to those operating in the normal parathyroid. Although a reduction of calcium sensing receptor expression is probably involved in the poor inhibition of **PTH** release induced by $(\text{Ca}^{2+})_o$, this is not the only factor altering $(\text{Ca}^{2+})_o$ sensing in parathyroid adenomas, since tumours characterized by different *in vitro* sensitivity to $(\text{Ca}^{2+})_o$ showed similar CaSR levels. The low content of G proteins of the Gq subfamily might represent an additional alteration leading to a defective $(\text{Ca}^{2+})_o$ sensing.

L10 ANSWER 3 OF 7 BIOSIS COPYRIGHT 2001 BIOLOGICAL ABSTRACTS INC.

2000:77827 Document No.: PREV20000077827. Hypercalcemia and

parathyroid hormone related protein expression in cutaneous T-cell lymphoma. Obagi, Suzan; DeRubertis, Fred; Brown, Edis; Deng, Jau Shyong (1). (1) Veterans Affairs Medical Center, University Drive C, Pittsburgh, PA USA. International Journal of Dermatology, (Nov., 1999) Vol. 38, No. 11, pp. 859-861. ISSN: 0011-3059. Language: English. Summary Language: English.

AB A 64-year-old white man presented to our clinic with a 1-year history of a lesion fading and extensive erythematous plaques on his trunk and extremities sparing the palmar and plantar surfaces. Further evaluation revealed profound peripheral and internal lymphadenopathy without visceral involvement on computed tomography (CT) scan. A diagnosis of cutaneous T-cell lymphoma (CTCL) was made based upon a skin biopsy (Fig. 1) and a cell phenotype analysis that revealed the predominance of helper T cells with a helper/suppressor T-11 ratio of greater than 10. The patient failed systemic psoralen plus UVA therapy and developed a 20 lb weight loss, circulating atypical lymphocytes, and hypercalcemia. He was asymptomatic from the hypercalcemia. He was started on 60 mg/day of prednisone. The patient's hypercalcemia resolved within 4 days of steroid therapy only to recur with attempts to taper the steroid dose. Laboratory studies (Table 1) were significant for negative human T-cell lymphotropic virus type 1

(HTLV-1) **antibody**, calcium = 14 mg/dL (normal, 8.5-10.5 mg/dL), plasma **parathyroid hormone (PTH)** = 9 pg/mL (normal, 10-65 pg/mL), and **parathyroid hormone related peptide (PTH-rp)** = 1.7 pmol/L (normal, <1.3 pmol/L). Immunohistochemistry staining with rabbit anti-**PTH-rp**-34 **polyclonal antibody** (Peninsula Laboratory, Belmont, CA, USA) was performed to study the expression of **PTH-rp** in paraffin-embedded sections of skin from the patient, five patients with CTCL without hypercalcemia, two patients with squamous cell carcinoma, and one normal human skin control. The sections were deparaffinized, rehydrated gradually, quenched and treated with 1 mg/mL protease-1 for 10 min at room temperature, and used in the immunohistochemistry staining as described by Deng et al. (Deng JS, Brod BA, Saito F, Tharp MD. Immune-associated cells in basal cell carcinomas of skin. *J Cutan Pathol* 1996; 23: 140-146.) Peripheral blood leukocytes from the reported patient were also stained in this manner to assess **PTH-rp** expression of the atypical lymphocytes. There was minimal staining for **PTH-rp** in skin from the normal control as well as patients with mycosis fungoïdes without hypercalcemia (Fig. 2), and a slight increase in staining intensity for **PTH-rp** in specimens from squamous cell carcinoma. There was strong expression of **PTH-rp** in keratinocytes as well as the infiltrating cells in the skin from the reported patient (Fig. 3). The patient's peripheral blood leukocytes were negative for **PTH**-rp. This strongly indicates that the keratinocytes and abnormal lymphocytes in the involved skin of our patient synthesized and expressed **PTH-rp**, which was subsequently secreted or released, contributing to the elevated circulating **PTH-rp** level and hypercalcemia.

L10 ANSWER 4 OF 7 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

1997:144937 Document No.: PREV19979954416. **Parathyroid**

hormone, prostaglandin E-2, and 1,25-dihydroxyvitamin D-3 decrease the level of Na⁺-Ca²⁺ exchange protein in osteoblastic cells. Krieger, N. S.. Dep. Med., Box 675, Univ. Rochester Sch. Med., 601 Elmwood Avenue, Rochester, NY 14642 USA. *Calcified Tissue International*, (1997) Vol. 60, No. 5, pp. 473-478. ISSN: 0171-967X. Language: English.

AB We previously described Na⁺-Ca²⁺ exchange in osteoblastic rat osteosarcoma cells (UMR-106) and demonstrated that Na⁺-dependent Ca²⁺ transport was inhibited by 24-hour treatment of cells with **parathyroid hormone (PTH)**, prostaglandin E-2 (PGE-2), or 1,25(OH)₂D-3. To determine whether this inhibition of Na⁺-Ca²⁺ exchange is at the level of exchanger protein synthesis we have examined exchanger protein levels using immunoblot analysis. UMR-106 cells were treated for 24 hours with or without **PTH**, PGE-2, or 1,25(OH)₂D-3. Plasma membrane fractions (750 g) were obtained and proteins were separated by SDS-PAGE, transferred to nylon membranes, and immunoblotted with a **polyclonal antibody** to the canine cardiac Na⁺-Ca²⁺ exchanger. In rat cardiac membranes, we detected 125 and 75 kD bands, similar to findings for the canine exchanger. In the osteoblastic UMR cell membranes, a specific band was detected at 90 kD that disappeared after treatment of cells with **PTH**. Inhibition by **PTH** was dose dependent, was maximal with 10⁻⁷ M **PTH**, and required 16-24 hour treatment time. Similar inhibition was observed after a 24 hour treatment with 10⁻⁶ M PGE-2 or 10⁻⁶ M 1,25(OH)₂D-3. These results demonstrate the presence of a specific protein in UMR cells that cross-reacts with **antibody** directed against the cardiac Na⁺-Ca²⁺ exchanger. Thus, the previously reported inhibition of Na⁺-Ca²⁺ exchange activity by calcemic agents in osteoblasts appears to be due to regulation of exchanger protein levels in these osteoblastic cells.

L10 ANSWER 5 OF 7 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

1995:33222 Document No.: PREV1995047522. Development of a sensitive two-site immunoassay for **parathyroid hormone**-related peptide: Evidence for elevated levels in plasma from patients

with adult T-cell leukemia/lymphoma and B-cell lymphoma. Ikeda, Kyoji (1); Ohno, Hideto; Hane, Motomu; Yokoi, Hiroyuki; Okada, Masato; Honma, Tamotsu; Yamada, Akiko; Tatsumi, Yoshiko; Tanaka, Tsuyoshi; Saitoh, Takao; Hirose, Sachio; Mori, Seiko; Takeuchi, Yasuhiro; Fukumoto, Seiji; Terukina, Shigeharu; Iguchi, Haruo; Kiriya, Takeshi; Ogata, Etsuro; Matsumoto, Toshiro. (1) Div. Endocrinol., Fourth Dep. Internal Med., Univ. Tokyo Sch. Med., 3-28-6 Mejirodai, Bunkyo, Tokyo 112 Japan. *Journal of Clinical Endocrinology & Metabolism*, (1994) Vol. 79, No. 5, pp. 1322-1327. ISSN: 0021-972X. Language: English.

AB We have developed a sensitive immunoradiometric assay for **PTH**-related peptide (PTHrP) using a monoclonal **antibody** against PTHrP(1-34) and a **polyclonal antibody** against PTHrP(50-83), with recombinant human PTHrP(1-87) as the standard. The detection limit of the immunoradiometric assay was 0.5 pmol/L, and plasma PTHrP(1-87) concentrations in 110 healthy subjects were 0.8 ± 0.5 pmol/L, with the upper limit of the normal range being 1.1 pmol/L. Increased circulating PTHrP(1-87) concentrations were demonstrated in all 46 cancer patients with hypercalcemia, but not in patients with primary hyperparathyroidism, chronic renal failure, or hypoparathyroidism. Normalization of serum calcium levels after resection of tumors was shown to correlate well with that of plasma PTHrP(1-87) concentrations in 2 cancer patients. High circulating PTHrP(1-87) levels were also demonstrated in 12 out of 13 hypercalcemic patients with adult T-cell leukemia/lymphoma and in 7 out of 8 hypercalcemic patients with non-Hodgkin's lymphoma especially of B-cell type. These results suggest that PTHrP is a major humoral factor responsible for the hypercalcemia frequently associated with adult T-cell leukemia/lymphoma and also with B-cell lymphoma.

L10 ANSWER 6 OF 7 BIOSIS COPYRIGHT 2002 BILOGICAL ABSTRACTS INC.
1994:58057 Document No.: PREV199497071657. Regulation of proliferation in JEG-3 cells by a 500-kDa Ca-2+ sensor and **parathyroid hormone**-related protein. Hellman, Per (1); Hellman, Bjorn; Juhlin, Claes (1); Juppner, Harald; Rastad, Jonas; Rudefelt, Peter; Akerstrom, Goran. (1) Dep. Surgery, Univ. Hosp., S-751 85 Uppsala Sweden. *Archives of Biochemistry and Biophysics*, (1993) Vol. 307, No. 2, pp. 379-385. ISSN: 0003-9861. Language: English.

AB JEG-3 cells are derived from human trophoblasts and demonstrated to express a 500-kDa Ca-2+ sensing protein, which elicits biphasic elevations of cytoplasmic Ca-2+ concentrations ([Ca-2+]i) and mediates Ca-2+ regulation of **parathyroid hormone**-related protein (PTHrP) release from placental cytotrophoblasts. Cytocentrifuged JEG-3 cells were immunostained by monoclonal and **polyclonal** antiserum toward PTHrP(1-34) and (35-64). Elevation of external Ca-2+ from 0.5 to 3.0 mM induced only a sluggish rise in [Ca-2+]i and no stimulation of cAMP production despite a more than twofold elevation of PTHrP(1-34) release. Monoclonal **antibodies** recognizing functionally discrepant epitopes of the Ca-2+ sensor protein substantiated uncoupling of this sensor in the Ca-2+-regulated PTHrP release. Exogenous activation of protein kinase C by a phorbol ester strongly augmented the secretion of PTHrP(1-34), whereby uncoupling of the Ca-2+ sensor was partially reversed. This functional differentiation was associated with reduced [³H]thymidine incorporation in JEG-3 cells. Proliferation of these cells was inhibited by 71% upon rise of extracellular Ca-2+ from 0.5 to 3.0 mM, and this inhibition was abolished by **antibody**-mediated interference with the Ca-2+ sensor function. PTHrP(1-34) and **PTH**(1-34) at concentrations up to 10⁻⁷ M decreased proliferation and stimulated the cAMP content of JEG-3 cells. The findings support concomitant Ca-2+ sensor and **PTH/PTHrP** receptor expression in JEG-3 cells, and that Ca-2+ inhibits proliferation by actions on the Ca-2+ sensor as well as by stimulation of PTHrP release possibly mediating autocrine growth inhibition.

1991:226819 Document No. 114:226819 Immunological identification and distribution of **parathyroid hormone**-like protein polypeptides in normal and malignant tissues. Kramer, Steven; Reynolds, Frederick H., Jr.; Castille, Macarena; Valenzuela, David M.; Thorikay, Midory; Sorville, John M. 'Oncogene Sci., Inc., Manhasset, NY, 11030, USA). Endocrinology (Baltimore), 128(4), 1927-37 (English) 1991. CODEN: ENDOAO. ISSN: 0013-7227.

AB Monoclonal and **polyclonal antibodies** recognizing human **parathyroid hormone**-like protein (PTHLP) have been produced using a series of recombinant and synthetic PTHLP peptides. These **antibodies** have been used to develop a two-site immunoassay which detects PTHLP[1-87] and PTHLP[1-141] but not **PTH**. The immunoassay detected PTHLP in exts. of squamous carcinomas and normal tissues at concns. from 7-515 ng PTHLP[1-87]/mg protein. Immunoblotting of the ext. which showed the highest immunoreactivity, a squamous carcinoma of the lung from a patient with hypercalcemia, revealed a major band of mol. wt. 26,500 and several other higher mol. wt. bands. Similar polypeptides were obsd. by immunoblotting cell exts. from a cell line, SCaBER, which secretes immunoreactive PTHLP into its medium and also from tumors in nude mice derived from this cell line. Chaotropic agents did not alter the immunoblotting pattern, and **antibodies** to three different epitopes of PTHLP recognized these bands, indicating PTHLP expression in the exts. Immunohistochem. staining of normal human tissue with these **antibodies** revealed several PTHLP-contg. tissues and confirmed the results of the immunoassay, suggesting a paracrine role for PTHLP. Staining was obsd. in several neoplastic tissues including squamous cell carcinomas, lung carcinoma, bladder carcinoma, osteogenic sarcoma, and adenocarcinoma of the colon.

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(FILE 'HOME' ENTERED AT 15:34:13 ON 22 SEP 2002)

FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 15:34:23 ON 22 SEP 2002

L1 234833⁰ S ANTIBODY
 L2 3431 S LI AND PARATHYROID HORMONE
 L3 1554 S L2 AND PTH
 L4 137 S LI AND HUMANS
 L5 1 S L4 AND DOGS
 L6 101 DUP REMOVE L4 (36 DUPLICATES REMOVED)
 L7 11 S L6 AND MONOCLONAL
 L8 20 DUP REMOVE L7 (3 DUPLICATES REMOVED)
 L9 7 S L6 AND POLYCLONAL
 L10 7 DUP REMOVE L9 (0 DUPLICATES REMOVED)

=> s 13 and bovine
 L11 282 L6 AND BOVINE

=> s 111 and monoclonal
 L12 36 L11 AND MONOCLONAL

=> s 112 and polyclonal
 L13 1 L12 AND POLYCLONAL

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 L14 1 DUP REMOVE L13 (3 DUPLICATES REMOVED)

=> d 114 cbik abs

L14 ANSWER 1 OF 1 MEDLINE DUPLICATE 1
94150234 Document Number: 94150234. PubMed ID: 8107517. E-cadherins
identified in osteoblastic cells: effects of **parathyroid**
hormone and extracellular calcium on localization. Babich M; Foti
L R. (Department of Biomedical Sciences, University of Illinois College of
Medicine, Rockford 61107-1897.) LIFE SCIENCES, (1994) 54 (11) PL201-8.
Journal code: 0375521. ISSN: 0024-3205. Pub. country: ENGLAND: United
Kingdom. Language: English.
AB The presence and regulation of cadherin localization in osteoblastic cells
were examined. **Monoclonal antibody** (ECCD-1) that
interferes with E-cadherin function prevented cell adhesion in UMR 106-H5
rat osteosarcoma cells and non-tumorigenic mouse calvarial MC3T3-E1 cells,
whereas CCL39 fibroblast adhesion was not affected. Immunofluorescent
antibodies (ECCD-2 and **polyclonal** L-CRMP P1) revealed
cadherins are localized along the osteoblastic cell-cell boundaries.
Exposure of UMR 106-H5 cells to **bovine parathyroid**
hormone (1-84) (**PTH**; 10 ng/ml x 1 hr) or low calcium
medium (1.0-0.025 mM) produced cellular retraction accompanied by intense
immunofluorescence for cadherins throughout cells with a corresponding
loss of punctate localization at remaining cell-cell adhesion points.
Western blot analysis indicated 108 kd and 115 kd cadherins are
present, with a smaller 29.5 kd band that became predominantly associated
with the cytosolic fraction of cells treated with **parathyroid**
hormone or lowered calcium. The results demonstrate E-like
cadherins are present in osteoblastic cells and implicate a regulatory
role for **parathyroid hormone** and calcium in cadherin
function and localization.

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PROCESSING COMPLETED FOR L12
L15 20 DUP REMOVE L1_ (36 DUPLICATES REMOVED)

=> d 115 1-20 cbib abs

L15 ANSWER 1 OF 20 CAPLUS COPYRIGHT 2002 ACS
2000:573647 Document No. 177:182977 Oxadiazole compositions for drug
delivery. Gschneidner, David (Emisphere Technologies, Inc., USA). PCT
Int. Appl. WO 2000047188 A1 20000817, 35 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, HF, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KE, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NC, NL, PT, PL, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UA, UZ, VN, YU, ZA, ZW, AM, AS, BY, KG, KH, MD, RU, TJ, TM; PW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, MD, ME, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIKKD2. APPLICATION:
WO 2000-US3899 20000211. PRIORITY: US 1999-PV119638 19990211.

AB Oxadiazoles and compns. for the delivery of active agents are provided.
Methods of administration and prepn. are provided as well. The
effectiveness of the oxadiazoles in increasing the serum levels of
second-mill human growth hormone and in calcin delivery of this hormone and other active agents was demonstrated.

L15 ANSWER 2 OF 20 SCISEARCH COPYRIGHT 2002 ISI (R)
1999:588016 The Genuine Article ID Number: 242MB. Effects of rat feline in
simulation of bone reabsorption in the presence of **parathyroid**
hormone. Nakamura O; Kazi J A; Ohnishi T; Arakaki N; Shao Q;
Kajihara T; Daikuhara Y (Reprint). KAGOSHIMA UNIV, SCH DENT, DEPT BIOCHEM,
57-1 SAKURAGAOKA 8, KAGOSHIMA 8908544, JAPAN (Reprint); KAGOSHIMA UNIV,
SCH DENT, DEPT BIOCHEM, KAGOSHIMA 8908544, JAPAN. BIOSCIENCE BICTECHNOLOGY
AND BIOCHEMISTRY (AUG 1999) V:1, 63, No. 8, pp. 1383-1391. Publisher:
JAPAN SOC BIOSCI BIOCITECHN AGRICHEM. JAPAN ACAD SOC CTR BLDG, 2-4-6 YAYOI

BUNKYO-KU, TOKYO 113, JAPAN. ISSN: 0916-8451. Pub. country: JAPAN.

Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Rat fetuin, which is the rat counterpart of human alpha(2)-HS glycoprotein and **bovine** fetuin, is only detectable in calcified tissues such as bone matrices and dentin, and bone cells such as osteoblasts and osteocytes immunohistochemically. The effect of this protein on bone resorption was examined to study its physiological role in bone metabolism. Rat fetuin increased bone resorption in the presence of low concentrations of **parathyroid hormone (PTH)**, but it had no activity on bone resorption without **PTH**. The increase in bone resorption by **PTH** and **PTH** plus rat fetuin was inhibited by the addition of chymostatin, an inhibitor for cathepsin L. Moreover, we found that when type I collagen from rat was preincubated with rat fetuin, the digestion of rat type I collagen by cathepsin L was increased. These findings suggest that rat fetuin present in bone matrix is important in bone resorption.

L15 ANSWER 3 OF 20 MEDLINE DUPLICATE 1
1998130566 Document Number: 98130566. PubMed ID: 9463353. Regulation of growth region cartilage proliferation and differentiation by perichondrium. Long F; Linsenmayer T F. (Department of Anatomy and Cellular Biology, Tufts University School of Medicine, Boston, MA 02111, USA.) DEVELOPMENT, (1998 Mar) 125 (6) 1067-73. Journal code: 8701744. ISSN: 0950-1991. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Endochondral bone formation in vertebrates requires precise coordination between proliferation and differentiation of the participating chondrocytes. We examined the role of perichondrium in this process using an organ culture system of chicken embryo tibiotarsi. A **monoclonal antibody** against chicken collagen type X, specifically expressed by hypertrophic chondrocytes, was utilized to monitor the terminal differentiation of chondrocytes. Proliferation of chondrocytes was examined by a BrdU-labeling procedure. The absence of perichondrium is correlated with an extended zone of cartilage expressing collagen type X, suggesting that the perichondrium regulates chondrocyte hypertrophy in a negative manner. Removal of perichondrium, in addition, resulted in an extended zone of chondrocytes incorporating BrdU, indicating that the perichondrium also negatively regulates the proliferation of chondrocytes. Partial removal of perichondrium from one side of the tibiotarsus led to expansion of both the collagen type X-positive domain and the BrdU-positive zone at the site of removal but not where the perichondrium remained intact. This suggests that both types of regulation by the perichondrium are local effects. Furthermore, addition of **bovine parathyroid hormone (PTH)** to perichondrium-free cultures reversed the expansion of the collagen type X-positive domain but not that of the proliferative zone. This suggests that the regulation of differentiation is dependent upon the **PTH/PTHRP** receptor and that the regulation of proliferation is likely independent of it. Taken together, these results are consistent with a model where perichondrium regulates both the exit of chondrocytes from the cell cycle, and their subsequent differentiation.

L15 ANSWER 4 OF 20 SCISEARCH COPYRIGHT 2002 ISI (R)
1998:458-51 The genuine Article (R) Number: 2T511. Vitamin D receptor gene polymorphism and parathyroid calcium sensor protein (CAS/PP330) expression in primary hypoparathyroidism. Farling T (Reprint); Pidefeldt B; Hellman B; Juhlin C; Lindgren E; Akreström S; Fastad J. UNIV UPPSALA HOSP, DEPT SURG, S-75185 UPPSALA, SWEDEN (Reprint); UNIV UPPSALA HOSP, DEPT CLIN CHEM, S-75185 UPPSALA, SWEDEN. WORLD JOURNAL OF SURGERY (JUL 1998) V.1. 12, No. 7, pp. 700-707. Publisher: SPRINGER VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010. ISSN: 0364-2813. Pub. country: SWEDEN. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Calcitriol, via its receptor (VDR), inhibits **parathyroid**

hormone (PTH) secretion and cell proliferation. physically linked polymorphic MPI alleles denoted b, a, and T, comprise a novel risk factor for postmenopausal primary hyperparathyroidism (pHPT) by their presumed coupling to reduced VDR expression. This study examines VDR gene polymorphisms, parathyroid calcium-regulated cytoplasmic calcium concentrations ($[Ca^{2+}]_{i}$) and parathyroid expression of a calcium sensor protein (CAS/gp330). Genomic DNA was obtained from 66 postmenopausal women with pHPT and 66 age-matched female controls. Polymorphic MDR alleles were detected after polymerase chain reaction (PCR) and restriction digestion. Cryosections of pathologic parathyroid glands from 41 of the patients were immunostained with a **monoclonal anti-CAS/gp330 antibody**. Heterozygosity for the VDR alleles b, a, and T was overrepresented in pHPT ($p < 0.01-0.05$) but did not couple to ED50 for calcium-regulated $[Ca^{2+}]_{i}$. The enlarged parathyroid glands possessed heterogeneous down-regulation of CAS/gp330. This down-regulation was the least conspicuous in the BE genotype and these few patients generally had larger parathyroid lesions ($p < 0.05$). The VEF haplotype bWT is a risk factor for pHPT possibly by hampering the regulatory actions of calcitriol. In contrast the BAt haplotype seems to be underrepresented in pHPT and to couple to larger parathyroid lesions as well as less deranged CAS/gp330 expression and parathyroid cell function. HPT in these individuals may relate to genetic events principally altering the regulation of cell proliferation, rather than calcium sensing of the parathyroid cells.

L15 ANSWER 5 OF 20 MEDLINE DUPLICATE 2
97006561 Document Number: 97006561. PubMed ID: 8653854. Effects of calcitonin and **parathyroid hormone** on the distribution of F-actin in the clear zone of osteoclasts in vivo. Kuroda M; Nakamura M; Kamiyama K. (Department of Pediatric Dentistry, School of Dentistry, Tohoku University, Sendai, Japan.) BONE, (1996 Aug 19; 21:115-20. Journal code: 8504048. ISSN: 0756-3282. Pub. country: United States. Language: English.

AB To elucidate the relation between the distribution of F-actin bands in the clear zone and the bone-resorbing activity of osteoclasts in vivo, the endocranial surfaces of calvariae from 7-day-old Wistar rats were stained with F-actin specific fluorescein isothiocyanate-labeled phalloidin (FITC-phalloidin) with and without the intraperitoneal injection of the calcium-regulating hormones, calcitonin (CT), and **parathyroid hormone (PTH)**. Some specimens were double-stained with FITC-phalloidin and a **monoclonal antibody**, ED1, which stained monocytes and macrophages. In normal rats, almost 80% of the osteoclasts showed ring-shaped F-actin bands in the clear zone, and the remaining 20% showed arch- or line-shaped F-actin bands. From 15 min to 1 h after the injection of CT (salmon, 10 mg/kg), the number of osteoclasts with ring-shaped F-actin bands decreased significantly. At 1 h, few F-actin bands were detected in osteoclasts, which were still stained by ED1. However, these F-actin bands recovered to the normal level at 6 h. On the other hand, from 15 min to 6 h after the injection of PTH (bovine, 50 mg/kg), the number of osteoclasts with arch- or line-shaped F-actin bands decreased significantly. These results indicate that osteoclasts with arch- or line-shaped F-actin bands in the clear zone had lower bone-resorbing activity than those with ring-shaped F-actin bands, and that the formation of bands could be controlled by calcium regulating hormones over the course of a few hours. Observation of the endocranial surfaces of calvariae might be useful for examining factors which affect the activities of osteoclasts in vivo.

L15 ANSWER 6 OF 20 MEDLINE DUPLICATE 3
95393875 Document Number: 95393875. PubMed ID: 7664644. Agonist-stimulated phosphorylation of the G protein-coupled receptor for **parathyroid hormone (PTH)** and **PTH**-related protein. Blind E; Bambino T; Nissenbaum R A. (Endocrine Unit, Veterans Administration Medical Center, San Francisco, California 94121,

USA.) ENDOCRINOLOGY, (1995 Oct) 136 (10) 4271-7. Journal code: 0375040.
ISSN: 0013-7227. Pub. country: United States. Language: English.

AB The objectives of the present study were to determine whether the G protein-coupled receptor for **PTH** and **PTH**-related protein (PTHRP) is subject to agonist-specific phosphorylation and to characterize the relevant kinase(s). The opossum kidney **PTH**/PTHRP receptor stably expressed in human embryonic kidney 293 cells was coupled to adenylyl cyclase, with half-maximal activation occurring in the presence of 0.1 nM **bovine** (b) **PTH**-(1-34). Immunoprecipitation of extracts of 32P-labeled cells using a **monoclonal antibody** to the **PTH**/PTHRP receptor revealed the presence of a major 32P-labeled protein of approximately 85 kilodaltons that was not evident in untransfected 293 cells. bPTH-(1-34) treatment produced a rapid dose-dependent increase in phosphorylation of the 85-kilodalton receptor, with a maximal effect that was 3.5 +/- 0.7-fold ($n = 4$; over basal). Half-maximal phosphorylation occurred with 10 nM bPTH-(1-34), similar to the hormone concentration required for 50% receptor occupancy. Activation of protein kinase A or protein kinase C with forskolin or phorbol 12-myristate 13-acetate also increased **PTH**/PTHRP receptor phosphorylation, but to a lesser degree than **PTH**. Neither of these kinases mediated the effect of **PTH**, as blockade of the protein kinase A pathway (with H-89) or the protein kinase C pathway (with the bisindolylmaleimide GF 109203X) did not inhibit bPTH-(1-34)-induced **PTH**/PTHRP receptor phosphorylation. These results suggest that agonist-stimulated **PTH**/PTHRP receptor phosphorylation may involve a nonsecond messenger-activated kinase, such as a member of the G protein-coupled receptor kinase family.

L15 ANSWER 7 OF 20 CAPLUS COPYRIGHT 2002 ACS
1995:860951 Document No. 123:276217 Homolog-scanning mutagenesis of the **parathyroid hormone** (**PTH**) receptor reveals **PTH**-(1-34) binding determinants in the third extracellular loop.
Lee, ChenWei; Luck, Michael D.; Jueppner, Harald; Pitts, John T., Jr.; Kronenberg, Henry M.; Gardella, Thomas J. (Endocrine Unit, Massachusetts General Hospital Harvard Medical School, Boston, MA, 02114, USA). Molecular Endocrinology, 9(10), 1269-78 (English) 1995. CODEN: MOENEN.
ISSN: 0888-8809. Publisher: Endocrine Society.

AB To identify determinants in the rat **PTH** receptor crit. for binding the agonist peptide, **PTH**-(1-34), the authors systematically replaced 12 segments (5-33 residues) of the receptor's extracellular surface with the corresponding segments of the homologous rat secretin receptor and screened the resulting mutants in COS-7 cells for altered **PTH**-(1-34) binding properties. Surface expression of mutant receptors was assessed by the binding of **monoclonal antibody** 12CA5 to the epitope (HA)-tagged receptors. Of the none well expressed and therefore informative receptor mutants, four bound radiolabeled **PTH**-(1-34) at levels that were proportional to the corresponding levels of surface expression, whereas five mutants bound [¹²⁵I]**PTH**-(1-34) to levels that were lower than predicted from the cell surface expression levels. These five mutations occurred at the extracellular (EC) end of transmembrane domain 1, the carboxy-terminal portion of the first EC loop, the second EC loop, and the third EC loop. The authors selected for further fine structure anal. the third EC loop; two specific residues, Trp-437 and Sln 440, were identified at which mutations caused 9- to 16-fold reduct. in **PTH**-(1-34)-binding affinity. The same mutations had little or no effect on the binding affinity of **PTH**-(1-34). This study provides new information on the location of **PTH** receptor regions important for high affinity agonist binding and identifies two residues in the third extracellular loop which may contribute to interactions involving the hormone's crit. amino terminus.

95009685 Document Number: 95009685. PubMed ID: 7523093. Synergistic effects of **parathyroid hormone** and 1,25-dihydroxyvitamin D3 on proliferation and vitamin D receptor expression of rat growth cartilage cells. Klaus G; von Eichel B; May T; Hugel U; Mayer H; Pitz E; Mehls O. (Department of Pediatrics, University of Heidelberg, Germany.) ENDOCRINOLOGY, (1994 Oct) 135 (4) 1307-15. Journal code: 0375040. ISSN: 0013-7227. Pub. country: United States. Language: English.

AB We investigated possible interaction of 1,25-dihydroxyvitamin D3 [1,25-(OH)2D3] and **PTH** on: 1) proliferation (monolayer culture) and colony formation (agarose stabilized suspension cultures); 2) expression of 1,25-(OH)2D3 receptor (VDR); and 3) cAMP response to **PTH**, using primary cultures of chondrocytes from rat tibia proximal epiphysis. 1 alpha,25-(OH)2D3 stereospecifically stimulated DNA synthesis, cell counts, and colony formation at low concentration (10(-12) M). Within 6 h **bovine PTH** (bPTH(1-34)), human **PTH** (hPTH(1-48)) (10(-10) M), cAMP (1-2 mM), and 12-O-tetradecanoyl-13-acetate (10(-8) M) increased [³H]thymidine incorporation in the absence and presence of 1,25-(OH)2D3. Both **PTH** fragments also stimulated chondrocyte growth and colony formation in a Ca dependent fashion. Prolonged exposure to bPTH(1-34) or hPTH(1-48) did not affect baseline DNA synthesis but increased the stimulatory effect of 1,25-(OH)2D3. This increase was inhibited in the presence of H7 (inhibition of PKC or the **monoclonal hPTH(1-38) antibody** A1-70). In subconfluent chondrocyte cultures VDR was up-regulated by bPTH(1-34) and hPTH(1-48) (10(-10) M) or activators of protein kinase C (PKC), but not by 8-Br-cAMP. It was blocked by cycloheximide and actinomycin D and persisted in the presence of Ca-channel blockers. Inhibition of PKC by H7 also blocked the effect of bPTH(1-34) on VDR. The cAMP response to bPTH(1-34) was not affected by 1,25-(OH)2D3. We conclude that: 1) DNA synthesis, cell proliferation, and colony formation in chondrocyte monolayer or suspension cultures is increased by aminoterminal and midregional **PTH** fragments and by cAMP analogs in a Ca- dependent fashion; 2) bPTH(1-34) and hPTH(1-48) up-regulate VDR by cAMP-independent, PKC-dependent steps requiring transcriptional and translational processes; both **PTH** fragments also amplify the effect of 1,25-(OH)2D3 on DNA synthesis; and 3) no difference is found between the bPTH(1-34) and hPTH(1-48) fragments with respect to chondrocyte proliferation and VDR up-regulation, although the two differ with respect to stimulation of cAMP production.

L15 ANSWER 9 OF 2 MEDLINE DUPLICATE 5
94150234 Document Number: 94150234. PubMed ID: 8107517. E-cadherins identified in osteoclastic cells: effects of **parathyroid hormone** and extracellular calcium on localization. Bakish M; Foti L R. (Department of Biomedical Sciences, University of Illinois College of Medicine, Rockford 61107-1877.) LIFE SCIENCES, (1994) 54 (11) P1201-5. Journal code: 0375021. ISSN: 0024-3205. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The presence and regulation of cadherin localization in osteoclastic cells were examined. **Monoclonal antibody** (MECD-1) that interferes with E-cadherin function prevented cell adhesion in UMR 10S-H5 rat osteosarcoma cells and non-tumorigenic mouse calvarial MC3T3-E1 cells, whereas MC3T3 fibroblast adhesion was not affected. Immunofluorescent antibodies (MECD-2 and polyclonal L-cAMP PI) revealed cadherins are localized along the osteoclastic cell-cell boundaries. Exposure of UMR 10S-H5 cells to **bovine parathyroid hormone** (1-84) (**PTH**; 10 ng/ml x 1 hr) or low calcium medium (1.0-0.025 mM) produced cellular retraction accompanied by intense immunofluorescence for cadherins throughout cells with a corresponding loss of punctate localization at remaining cell-cell adhesion points. Western immunoblot analysis indicated 108 kd and 115 kd cadherins are present, with a smaller 29.5 kd band that became predominantly associated with the cytosolic

fraction of cells treated with **parathyroid hormone** or lowered calcium. The results demonstrate E-like cadherins are present in osteoblastic cells and implicate a regulatory role for **parathyroid hormone** and calcium in cadherin function and localization.

L15 ANSWER 10 OF 20 MEDLINE

95053517 Document Number: 95053517. PubMed ID: 7964284. Binding of 1,25-dihydroxyvitamin D3 receptors to the 5'-flanking region of the **bovine parathyroid hormone** gene. Hawa N S; O'Riordan J L; Farrow F M. (Department of Medicine, University College London Medical School, Middlesex Hospital, UK.) JOURNAL OF ENDOCRINOLOGY, (1994 Jul) 142 (1) 53-60. Journal code: 0375363. ISSN: 0022-0795. Pub. country: ENGLAND: United Kingdom. Language: English.

AB To further define the binding site for receptors for 1,25(OH)₂D₃ (VDR) in the **bovine PTH** gene and to study the interactions of transcription factors with VDR, Southwestern and gel shift assays were used. Data from the former indicated binding of VDR to DNA fragments spanning the regions -451 to -348 bp and -668 to -452 bp. Studies using gel shift assays confirmed binding to the -451 to -348 bp fragment and specificity was shown by using excess concentrations of unlabelled -451 to -348 bp fragment to compete for binding, whereas excess unlabelled -347 to +50 bp did not compete. Binding was also observed with the -668 to -452 bp fragment but excess concentrations of unlabelled -668 to -452 or -451 to -348 bp fragments did not compete for binding to radiolabelled fragments. These data indicate the presence of two binding domains within this region; the upstream element having a lower affinity for VDR than the downstream element. In addition, there was no interaction between VDR and consensus sequences for AP1, AP2, APE and SP1. The putative vitamin D₃ response element (VDRE) contains two similar hexameric steroid response element-like half-sites placed as AGGTCA-related direct repeats. The upstream repeat is at -461 to -456 bp and the downstream element is at -449 to -444 bp. The presence of these half-sites is consistent with our experimental data in which cleavage with SspI at -452 bp resulted in two DNA fragments which bound VDR.

L15 ANSWER 11 OF 20 SCISEARCH COPYRIGHT 2002 ISI (R)

93:304962 The Genuine Article (R) Number: LA850. CHARACTERIZATION OF **BOVINE** OSTEOCLASTS ON AN IONOMERIC CEMENT INVITRO. SZULCZEWSKI D H; MEYER U; MOLLER K; STRATMANN U; DOTY S B; JONES D B (Reprint). UNIV MUNSTER, INST ANAT, DENT SECT, CELL BIOL EXPTL ORTHOPAED LAB, DOMAGKSTR 3, W-4400 MUNSTER, GERMANY; HOSP SPECIAL SURG, NEW YORK, NY, 10031. CELLS AND MATERIALS (1993) Vol. 1, No. 1, pp. 33-32. ISSN: 1061-6784. Pub. country: GERMANY; USA. Language: ENGLISH.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Primary **bovine** osteoclasts were obtained by an outgrowth method from **bovine** periosteum and cultured for 7 days on an ionomeric cement for biomaterial testing. Osteoclasts cultured on slices of **bovine** bone and on glass microscope cover-slides served as a control. The cells were characterised as osteoclasts by a number of tests. Osteoclasts showed positive staining for tartrate resistant acid phosphatase and reactivity with the **antibodies** IgG and IgM, which react with the alpha-chain of the vitronectin receptor. Addition of salmon calcitonin to the culture medium led to sudden cessation of lamellipodial activity. The cells resorbed bone by mixing pit. In mixed cultures with osteoblasts, the morphology of the osteoclasts on the smooth ionomeric cement surface was comparable to that on glass cover slides, revealing broad cytoplasmatic extensions on the material. Acridine orange staining demonstrated viability of cells until the end of the culture period and increased acidification after **parathyroid hormone** (**PTH**) stimulation. Scanning electron microscopy did not reveal erosion of the material by osteoclasts. No signs of aluminium toxicity on osteoclasts could be detected during the 7 day culture period, although an increased uptake of aluminium into the cell

was demonstrated.

L15 ANSWER 12 OF 20 MEDLINE

92059485 Document Number: 92059485. PubMed ID: 1659224. Active Ca²⁺ transport in primary cultures of rabbit kidney CCD: stimulation by 1,25-dihydroxyvitamin D₃ and **PTH**. Bindels R J; Hartog A; Timmermans J; Van Os C H. (Department of Physiology, University of Nijmegen, The Netherlands. : AMERICAN JOURNAL OF PHYSIOLOGY, (1991 Nov) 261 (5 Pt 2) F799-807. Journal code: 0370511. ISSN: 0002-9513. Pub. country: United States. Language: English.

AB Rabbit connecting tubules and cortical collecting ducts, which represent 79 +/- 5% of the calbindin-D28k-containing kidney cells, were isolated by immuno dissection from the rabbit kidney superficial cortex and seeded on permeable filters. After 6 days in culture the monolayers developed a potential difference (PD) of -24 +/- 1 mV lumen negative and a transepithelial resistance (R) of 234 +/- 13 omega.cm². Addition of 10(-6) M amiloride to or removal of Na⁺ from the mucosal side reversed the PD to +6 +/- 4 mV and concomitantly increased R to 660 +/- 122 omega.cm². The cells developed functional **parathyroid hormone** (**PTH**) and arginine vasopressin receptors, but calcitonin receptors were absent. The monolayer actively absorbed Ca²⁺ against an electrochemical gradient with a rate of 121 +/- 13 nmol.h-1.cm⁻². Removal of serosal Na⁺ inhibited Ca²⁺ absorption by 63 +/- 3%. Exposure to 10(-7) M 1,25-dihydroxyvitamin D₃ [1,25(OH)D₃] for 48 h or 10(-7) M **bovine PTH** (bPTH)-(1-34) for 1 h, increased transcellular Ca²⁺ absorption by 53 +/- 15% or 24 +/- 8%, respectively. The effects of 1,25(OH)D₃ and **PTH** in combination were neither additive nor potentiating. In addition, the cultured cells expressed calbindin-D28k, and, after exposure to 10(-7) M 1,25(OH)D₃ for 48 h, the calbindin-D28k content increased four-fold.

L15 ANSWER 13 OF 20 MEDLINE

DUPLICATE 6

9101119 Document Number: 91011219. PubMed ID: 170554. Binding of the receptor for 1,25-dihydroxyvitamin D₃ to the 5'-flanking region of the **bovine parathyroid hormone** gene. Farrow S M; Hawa N S; Karmali R; Hewison M; Walters J C; O'Farrell J L. (Department of Medicine, Middlesex Hospital, London.) JOURNAL OF ENDOCRINOLOGY, (1990 Sep) 126 (Pt 3) 355-9. Journal code: 0375363. ISSN: 0022-0795. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Receptors for 1,25-dihydroxyvitamin D₃ (1,25-(OH)D₃) were prepared from **bovine** parathyroid glands and incubated with fragments of DNA of the 5'-flanking region of the **bovine parathyroid hormone** (**PTH**) gene covering 1700 base pairs (bp) upstream of the initiation site. In filter binding assays, incubation of the DNA fragment spanning -700 to +50 bp with 300 micrograms cytosolic protein gave 288 +/- 63% (mean +/- S.D.) of binding in the absence of protein. In contrast, there was no significant reaction with the -1350 to -700 bp fragment, nor was there binding of the receptor to a fragment of DNA covering the coding region of the **PTH** gene. Substitution of **bovine** serum albumin for the receptor preparation did not induce binding to the -700 to +50 bp fragment. The receptor binding site was further defined to -700 to -100 bp as deletion of the -100 to +50 bp did not reduce receptor binding. Reaction of receptors further purified by sucrose density ultracentrifugation with a **monoclonal antibody** in immunoblots revealed a single species with a molecular mass of approximately 100,000 Da, which was absent in preparations of cos-1 cells. Autoradiography following incubation of receptors immobilized on nitrocellulose filters with the -700 to +50 bp fragment indicated a single reactive band coincident with the band in the immunoblot. The DNA fragment did not bind to filters containing preparations of cos-1 cells. Extraction of the receptors in the presence or absence of 1,25-(OH)D₃ (4 nmol/l) or the presence of KCl (150 mmol/l) in the incubation medium had no significant effect on DNA binding to the protein in this assay. (ABSTRACT

TRUNCATED AT 250 WORDS)

L15 ANSWER 14 OF 20 MEDLINE DUPLICATE 7
90203631 Document Number: 90203631. PubMed ID: 1690780. Production and
characterisation of **monoclonal antibodies** to
parathyroid hormone-related protein. Patcliffe W A;
Hughes S; Gilligan M G; Heath D A; Patcliffe J G. (Wolfson Research
Laboratories, Department of Clinical Chemistry, Queen Elizabeth Medical
Centre, Birmingham, U.K.) JOURNAL OF IMMUNOLOGICAL METHODS, (1990 Feb 20)
127 (1) 109-16. Journal code: 1305440. ISSN: 0022-1759. Pub. country:
Netherlands. Language: English.
AB The production and characterisation of 17 **monoclonal**
antibodies to human **parathyroid hormone**
-related protein (**PTH**-rP 1-34) is described. Five of the
antibodies were shown to be of high avidity (Ka : X 10¹⁰ - 1.3 X
10¹¹ M⁻¹) and able to detect 15-180 pg **PTH**-rP 1-34 per tube by
PIA. None cross-reacted with **PTH** 1-34, and inhibition studies
with peptide subfragments of **PTH**-rP 1-34 indicated that all
recognise a central region extending from residues 9-18 to between
residues 23 and 34. All **antibodies** tested cross-reacted with
native **PTH**-rP in culture fluids from keratinocytes and squamous
cancer cell lines and in human and **bovine** milk. The
concentrations of **PTH**-rP 1-34 (ng/ml) in these fluids as
determined by PIA were: keratinocytes 1-3, squamous cancer 0.2-2.5, human
milk, up to 80. Selected **antibodies** coupled to Sepharose 4B were
used to extract **PTH**-rP from biological fluids with high yields.

L15 ANSWER 15 OF 20 BIOSIS COPYRIGHT 1992 BIOLOGICAL ABSTRACTS INC.
1989:384466 Document No.: BR37:61114. **MONOCLONAL ANTIBODIES**
TO **PARATHYROID HORMONE PTH-RELATED PROTEIN**
FPP. PATCLIFFE W A; PATCLIFFE J G; GILLIGAN M G; HEATH D A. WOLFSON RES.
LAB., UNIV. DEF. CLIN. CHEM., BIRMINGHAM B15 2TH.. 8TH JOINT MEETING OF
BRITISH ENDOCRINE SOCIETIES, MANCHESTER, ENGLAND, UK, APRIL 10-13, 1989. J
ENDOCRINOL. (1989) 121 (SUPPL), NO PAGINATION. CODEN: JOENAK. ISSN:
0022-0795. Language: English.

L15 ANSWER 16 OF 20 MEDLINE DUPLICATE 8
89248106 Document Number: 89248106. PubMed ID: 3148228.
Monoclonal antibodies to **bovine**
parathyroid hormone: production and characterization.
Vieira J G; Federico P; Matsueda G; Neer R M. (Mineral Metabolism Unit,
Massachusetts General Hospital, Boston 02114.) BRAZILIAN JOURNAL OF
MEDICAL AND BIOLOGICAL RESEARCH, (1988) 21 (5): 1065-11. Journal code:
0100-879X. Pub. country: Brazil. Language: English.
AB 1. This paper describes the production and characterization of
monoclonal antibodies against **bovine**
parathyroid hormone (bPTH)-(1-34). 2. Spleen cells from
A/J mice successfully immunized with bPTH-(1-34) were fused with SP2/O
myeloma cells using PEG 4000 as fusogen. The screening method employed
microtiter plates coated with sheep antimouse IgG and the presence of
specific **monoclonal antibodies** was demonstrated by the
binding of ¹²⁵I-bPTH-(1-34). 3. A detailed study of the specificity of the
three viable **monoclonals** with highest affinity showed that two
(6F6 and 6D4) were amino-terminal specific and the other (5B9)
carboxy-terminal specific. The two amino-terminal **monoclonal**
antibodies appear to recognize the same carboxylic site. 4. The
monoclonal antibodies produced are potentially useful
reagents for the development of new methods for the measurement of
PTH in biological fluids, studies on the interaction of
PTH with its receptor, as well as localization of **PTH**
producing cells.

L15 ANSWER 17 OF 20 MEDLINE DUPLICATE 9

88134998 Document Number: 88134998. PubMed ID: 3342264. Modulation of the Ca²⁺-sensing function of parathyroid cells in vitro and in hyperparathyroidism. Nygren P; Gylfe E; Larsson P; Johansson H; Juhlin C; Klareskoq L; Akerstrom G; Pastad J. (Department of Medical Cell Biology, University of Uppsala, Sweden.) BIOCHIMICA ET BIOPHYSICA ACTA, (1988 Feb 22) 968 (2) 253-60. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.

AB When raising the extracellular Ca²⁺ concentration stepwise from 0.5 to 3.0 mM, **bovine** parathyroid cells reacted with initial transient and sustained elevations of the cytoplasmic Ca²⁺ concentration (Ca²⁺i), as well as more than 50% inhibition of **parathyroid hormone** (PTH) release. Human parathyroid adenoma cells and **bovine** cells cultured for 1 day or exposed to a low concentration of a **monoclonal** antiparathyroid **antibody** exhibited right-shifted dependencies of PTH release and Ca²⁺i on extracellular Ca²⁺ and reduced Ca²⁺i transients. The protein kinase C activator 12-O-tetradecanoylphorbol-13-acetate (TPA) further right-shifted the dose response relationship for Ca²⁺ regulated Ca²⁺i of the adenoma cells, whereas the protein kinase C inhibitor 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H-7) tended to normalize it, without affecting Ca²⁺i of normal **bovine** cells. In cells from an oxyphil adenoma and a parathyroid carcinoma as well as in **bovine** cells cultured 4 days or exposed to a high concentration of the antiparathyroid **antibody**, there were no Ca²⁺i transients, very small increases in steady-state Ca²⁺i and nonsuppressible PTH release. The results suggest that reduced availability of a putative Ca²⁺-receptor and increased protein kinase C activity may be important factors in the decreased Ca²⁺ sensitivity of abnormal parathyroid cells.

L15 ANSWER 18 OF 26 MELLINE DUPLICATE 19
85258588 Document Number: 85258588. PubMed ID: 2991044. Identification of a **monoclonal antibody** which interacts with the **parathyroid hormone** receptor-adenylate cyclase system in murine bone. Weinshank R L; Cain C D; Vasquez N B; Luben R A. MOLECULAR AND CELLULAR ENDOCRINOLOGY, (1985 Jul) 41 (2-3) 337-46. Journal code: 7500844. ISSN: 0303-7207. Pub. country: Netherlands. Language: English.

AB We have produced **monoclonal antibodies** which bind specifically to mouse bone cells and then selected these **monoclonal antibodies** for their ability to inhibit **parathyroid hormone** (PTH) responses in mouse cranial bone treated with the (1-34) amino terminal peptide of **bovine PTH** (bPTH(1-34)). One clone, designated 3-6, characterized as an IgM(kappa), significantly inhibited the accumulation of cAMP in response to bPTH(1-34) at concentrations of hormone between 10(-9) and 10(-7) M. This **antibody** was subsequently isolated by gel filtration and shown to bind to intact mouse calvariae, with saturation binding occurring at 3 micrograms/ml IgM. A maximal inhibition of approximately 70% of the cAMP accumulation produced in response to 2.5 X 10(-9) M (100 ng/ml bPTH 1-34) was obtained with 7 micrograms/ml of the purified 3-6 IgM. At this concentration of 3.6 IgM, the half-maximal dose of PTH for cAMP accumulation was increased from 5 X 10(-9) M to 2 X 10(-8) M with no reduction in maximal levels of cAMP production. The utility of this **antibody** as an inhibitor was further tested by its ability to block the binding of an iodinated PTH analogue, 125I-[Nle8, Nle18, Tyr34]-bPTH(1-34) to mouse cranial bone. The 3.6 IgM at a concentration of 10 micrograms/ml inhibited 70% of the specific binding of the iodinated analogue. In the absence of **parathyroid hormone**, 2 X 10(-8) M 3-6 IgM produced a 4-fold increase in cAMP above basal levels, as compared to 40-fold maximal increases observed with PTH, indicating a partial PTH agonist activity of this **antibody**. When tested for effects on other hormones, 3-6 IgM did not inhibit cAMP accumulation produced in response to salmon calcitonin, epinephrine, prostaglandin E2 or cholera

toxin. We propose that the 3-6 **monoclonal** IgM is specific for the **PTH** receptor or a component of the **PTH** receptor-adenylate cyclase system and that this or similar **antibodies** will serve as useful reagents for future molecular characterization of this receptor.

L15 ANSWER 19 OF 20 MEDLINE DUPLICATE 11
86055853 Document Number: 86055853. PubMed ID: 2998791. Identification of a 150-kDa membrane component which is modulated by **parathyroid hormone**. Weinshank R L; Luben R A. EUROPEAN JOURNAL OF BIOCHEMISTRY, (1985 Nov 15) 153 (1) 179-88. Journal code: 0107600. ISSN: 0014-2956. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.

AB **Monoclonal antibodies** have been produced against primary bone cells obtained from the collagenase digestion of mouse cranial bone. **Antibodies** were selected on the basis of their immunoglobulin class and those which were identified as IgG were further screened for their ability to inhibit cAMP accumulation in response to sub-maximal doses of the 1-34 amino-terminal peptide of **bovine parathyroid hormone**, bPTH(1-34). Nine hybridoma clones were subsequently characterized as inhibitory with respect to **parathyroid hormone** (PTH) responses in intact mouse cranial bone and which also identified a variety of membrane components from detergent extracts of surface-labeled primary bone cells. Five of these **antibodies** immunoprecipitated a membrane component with Mr of 80 000 that appeared to be a major component of the extract susceptible to surface-labeling with ^{125}I . All nine **monoclonal antibodies** were shown to bind to a suspended-cell preparation of primary bone cells with 2-3 orders of magnitude greater binding than that of control **antibodies**. Using this assay, one clone, designated 3G12 IgG, was observed to exhibit desensitization effects at the binding level with a time course and dose dependency for **PTH** pre-incubation that was similar to the establishment of the refractory state in other systems. In addition, the desensitization effect occurred at 37 degrees C but not at 4 degrees C. This **antibody** was shown to bind saturably to both intact mouse cranial bone and primary bone cells with an apparent affinity constant (K_a) in the range of 10(3) M. Inhibition of bone cAMP accumulation in response to 2.5 nM bPTH(1-34) was directly correlated to the binding of 3G12 IgG to intact mouse calvariae. A maximum inhibition of approximately 85% was observed. 3G12 IgG immunoprecipitated a single membrane component, Mr 150 000, from NP-40 detergent extracts of ^{125}I -labeled primary mouse bone cells. The molecular mass of this component was also 150 000 daltons when run on polyacrylamide gel slabs under non-reducing conditions. Control and **PTH**-pre-treated bone cells were surface-labeled, detergent-solubilized and immunoprecipitated with 3G12 IgG in order to investigate further the desensitization effect at the molecular level. Incubation of bone cells with 1 microgram/ml bPTH(1-34) for 45 min at 37 degrees C caused an increased susceptibility to surface-labeling with ^{125}I that was approximately three-fold higher in specific activity than that of control cells. ABSTRACT TRUNCATED AT 400 WORDS

L15 ANSWER 20 OF 20 CAPLUS COPYRIGHT 2001 ACS
1983:19613. Document No. 98:19613 Production and characterization of **monoclonal antibodies** specific for parathormone. Van de Walle, P.; Parfet, R.; Maron, J.; Maassen, J. B.; Urbain, J. (Inst. Natl. Radicelem., Fleurus, Belg.). Peptides Biol. Fluids, Volume Date 1982, 50, 555-7 (English) 1983. CODEN: PBFPA6. ISSN: 0079-7065.

AB Spleen cells from 2 mice of different strains, immunized with **bovine parathyroid hormone** (bPTH), have been fused with mouse myeloma cells. Hybridomas obtained were screened for secretion of **antibodies** specific for b-PTH using liq. and solid phase RIA. Pos. cultures were cloned in agar and by limiting

diln.; 13 specific **monoclonal** hybridomas were obtained. The classes of Ig's of the different **monoclonal antibodies** produced were detd.; IgG1, IgG2a, IgG2b, and IgM **antibodies** were found. Assocn. const. for ¹²⁵I-labeled b-**PTH** and antigenic specificities for 3 **monoclonals** were detd.

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FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 15:34:23 ON 22 SEP 2002

L1 2348554 S ANTIBODY
L2 3171 S L1 AND PARATHYROID HORMONE
L3 1771 S L1 AND PTH
L4 147 S L3 AND HUMANS
L5 1 S L4 AND DOGS
L6 161 DUP REMOVE L4 (36 DUPLICATES REMOVED)
L7 20 S L6 AND MONOCLONAL
L8 20 DUP REMOVE L7 (0 DUPLICATES REMOVED)
L9 7 S L6 AND POLYCLONAL
L10 7 DUP REMOVE L9 (0 DUPLICATES REMOVED)
L11 292 S L3 AND BOVINE
L12 56 S L11 AND MONOCLONAL
L13 4 S L12 AND POLYCLONAL
L14 1 DUP REMOVE L13 (0 DUPLICATES REMOVED)
L15 19 DUP REMOVE L12 (36 DUPLICATES REMOVED)

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L16 469 L3 AND RAT

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MISSING OPERATOR L16 ADN

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L17 46 L16 AND MICE

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L18 .4 DUP REMOVE L17 (11 DUPLICATES REMOVED)

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L18 ANSWER 1 OF 24 MEDLINE

2002070453 Document Number: 21655619. PubMed ID: 11796519. Estrogen inhibition of **PTH**-stimulated osteoclast formation and attachment *in vitro*: involvement of b-**PTH** PKA and PKC Liu Eu-Yuan; Wu Pei-Wen; Bringhurs; F. Fischbeck; Wang Teung-Tsung. School of Dentistry, National Taiwan University College of Medicine, Taipei, Taiwan 10016, Republic of China. *ENDOCRINOLOGY*, (2002 Feb) 143 (2): 627-35. Journal code: J:75140. ISSN: 0013-7227. Pub. country: United States. Language: English.

AB Estrogen modulates the stimulatory effects of **PTH** on bone *in vivo* and *in vitro*. **PTH**-stimulated cAMP accumulation in osteoblasts is thought to be linked to increased osteoclastic activity, but the precise mechanism is still unknown. In cocultures of clonal marrow stromal cells (MS1) and normal mouse spleen cells, both 1,25-dihydroxyvitamin D₃ and **rat PTH** (rPTH)-1-34 can induce the formation of tartrate-resistant acid phosphatase- and calcitonin receptor-positive multinucleated osteoclast-like cells, which can attach to dentine slices

and produce resorption pits. In this system, osteoclastogenesis stimulated by **PTH**, but not by 1,25-dihydroxyvitamin D₃, was suppressed by 17beta-E2 (10(-10)-10(-8) M), whereas 17alpha-E2 (10(-8) M) had no effect. Exposure to 10(-8) M 17beta-E2, but not 17alpha-E2, also significantly decreased the **PTH**-induced attachment of osteoclast-like cells to dentine slices. 17beta-E2 inhibited osteoclast-like cell formation induced by 8-kromo-cAMP (10(-4) M), 12-O-tetradecanoylphorbol 13-acetate (10(-8) M), or **rat PTH**-(1-34) (10(-7) M) in combination with either rp-adenosine-3',5'-cyclic monophosphorothioate (10(-4) M) or 1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride (10(-5) M). 17beta-E2 suppressed the partial stimulation of tartrate-resistant acid phosphatase-positive multinucleated osteoclast-like cell formation induced by [Arg(2)]human (h) **PTH**-(1-34) (10(-7) M) or hPTH-(3-34) (10(-7) M), but not that caused by 10(-7) M hPTH-(53-84). We conclude that estrogens suppress **PTH**-stimulated osteoclast-like cell formation by blocking both the cAMP-dependent PKA pathway and the PLC-coupled calcium/PKC pathway. In addition to inhibiting formation of osteoclasts and promoting their apoptosis, estrogen may regulate bone resorption by blocking attachment of osteoclasts to bone.

L18 ANSWER 2 OF 24 SCISEARCH COPYRIGHT 2002 ISI (R)
2001:783297 The Genuine Article (R) Number: 476PV. Evidence that anabolic effects of **PTH** on bone require IGF-I in growing **mice**.
Miyakoshi N; Kasukawa Y; Linkhart T A; Baylink D J; Mchan S (Reprint). JL Pettis Vet Affairs Med Ctr, Musculoskeletal Dis Ctr 151, 11201 Benton St, Loma Linda, CA 92357 USA (Reprint); JL Pettis Vet Affairs Med Ctr, Musculoskeletal Dis Ctr 151, Loma Linda, CA 92357 USA; Loma Linda Univ, Dept Physiol, Loma Linda, CA 92350 USA; Loma Linda Univ, Dept Pediat, Loma Linda, CA 92350 USA; Loma Linda Univ, Dept Biochem, Loma Linda, CA 92350 USA; Loma Linda Univ, Dept Med, Loma Linda, CA 92350 USA. ENDOCRINOLOGY (OCT 2001) Vol. 142, No. 10, pp. 4349-4356. Publisher: ENDOCRINE SOC. 4350 EAST WEST HIGHWAY SUITE 500, BETHESDA, MD 20814-4110 USA. ISSN: 0013-7227. Pub. country: USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Although it has been established that **PTH** exerts potent anabolic effects on bone in animals and humans, the mechanism of **PTH** action on bone remains controversial. Based on the previous findings that **PTH** treatment increased production of IGF-I in bone cells and that **PTH** effects on bone cells in vitro were blocked by IGF-I-blocking **antibodies**, we proposed that IGF-I action is required for the stimulatory effects of **PTH** on bone formation. To test this hypothesis, we evaluated the effects of **PTH** on bone formation parameters in growing **mice** lacking functional IGF-I genes. Five-week-old IGF-I(-/-) **mice** and wild-type littermates were given daily s.c. injections of 160 mug/kg body weight of **PTH** (1-34) or vehicle for 10 d. In wild-type animals, **PTH** caused a significant increase in serum osteocalcin levels (113%), serum alkaline phosphatase activity (43%), and alkaline phosphatase activity in femoral bone ex-tracts (30%), compared with the vehicle-treated control group. In contrast, in IGF-I(-/-) **mice**, there was no significant effect of **PTH** on any bone formation parameters. **PTH** treatment increased total bone mineral density, as evaluated by peripheral quantitative computer tomography, at the distal metaphysis of the femur by 40% in wild-type **mice**, but it had no effect on bone mineral density in **mice** lacking functional IGF-I genes. *In vitro* studies using osteoclasts derived from control and IGF-I(-/-) **mice** revealed that **PTH** treatment increased cell number in osteoblasts derived from IGF-I knockout **mice** in the presence of exogenously added IGF-I but not without IGF-I. These data to our knowledge provide the first direct evidence that the anabolic effects of **PTH** on bone formation *in vivo* require IGF-I action in growing **mice**.

L18 ANSWER 3 OF 24

2001212000 Document Number: 21065189. PubMed ID: 11120880. The chondrogenic transcription factor Sox9 is a target of signaling by the **parathyroid hormone**-related peptide in the growth plate of endochondral bones. Huang W; Chung U I; Krnecnberg H M; de Crombrugghe B. (Department of Molecular Genetics, University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, USA.) PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (2001 Jan 2: 98 (1) 160-5. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB In the growth plate of endochondral bones, **parathyroid hormone (PTH)**-related peptide (PTHrP) regulates the rate of chondrocyte maturation from prehypertrophic chondrocytes to hypertrophic chondrocytes. Using an **antibody** specific for Sox9 phosphorylated at serine 181 (S181), one of the two consensus protein kinase A phosphorylation sites of Sox9, we showed that the addition of PTHrP strongly increased the phosphorylation of Sox9 in COS7 cells transfected with both **SOX9**- and **PTH/PTHrP receptor**-expressing vectors. PTHrP also increased the **SOX9**-dependent activity of chondrocyte-specific enhancers in the gene for type II collagen (Col2a1) in transient transfection experiments. This increased enhancer activity did not occur with a **Sox9** mutant harboring serine-to-alanine substitutions in its two consensus protein kinase A phosphorylation sites. Consistent with these results, PTHrP also increased Col2a1 mRNA levels in **rat** chondrosarcoma cells as well as 10T1/2 mesenchymal cells transfected with a **PTH/PTHrP receptor** expressing plasmid. No phosphorylation of **sox9** at S181 was detected in prehypertrophic chondrocytes of the growth plate or any chondrocytes of **PTH/PTHrP receptor** null mutants. In contrast in wild-type mouse embryos, previous immunohistochemistry experiments indicated that **Sox9** phosphorylated at S181 was detected almost exclusively in chondrocytes of the prehypertrophic zone. **Sox9**, regardless of the phosphorylation state, was present in all chondrocytes of both genotypes except hypertrophic chondrocytes. Our results indicated that **Sox9** is a target of PTHrP signaling in prehypertrophic chondrocytes in the growth plate. We hypothesize that **Sox9** mediates at least some effects of PTHrP in the growth plate and that the PTHrP-dependent increased transcriptional activity of **Sox9** helps maintain the chondrocyte phenotype of cells in the prehypertrophic zone and inhibits their maturation to hypertrophic chondrocytes.

L18 ANSWER 4 OF 24 CACPLUS COPYRIGHT 2002 ACS

AB The present invention relates to studies of characterization of glucose dependent insulinotropic peptide (GIP), a commonly known gastrointestinal hormone for its role of coupling nutrient intake and insulin secretion, as an osteotropic hormone which can be used for treatment of bone deposition diseases. These studies provide the evidence that GIP receptors are present in bone and bone-derived cells like osteoblastic-like cells SaOS2 and that stimulation of these cells with GIP results in increase in intracellular calcium levels, cellular cAMP

content, type I collagen expression and alk. phosphatase activity. The physiol. role of GIP to modulate bone deposition is also demonstrated in animal models by showing its inhibitory effect on bone resorption in **rats** induced by **PTH** or ovariectomized, or showing increased bone d. in transgenic **mice** overexpressing GIP. To treat bone deposition diseases, GIP or analogs can be used to inhibit bone resorption and to maintain or increase bone d., while GIP antagonists and compds. blocking GIP binding to GIP receptor (like anti-idiotypic **antibody**) can be used to decrease bone d.

L18 ANSWER 5 OF 24 MEDLINE

DUPLICATE 1

2000354698 Document Number: 20354698. PubMed ID: 10898333.

Parathyroid hormone-related protein as a potential target of therapy for cancer-associated morbidity. Ogata E. (Japanese Foundation for Cancer Research, Tokyo.) *J CANCER*, 2000 Jun 15; 81 (12 Suppl): 1913-14. Journal code: 0021-4338. ISSN: 0168-543X. Pub. country: United States. Language: English.

AB BACKGROUND: Proinflammatory cytokines are involved in the genesis of cancer-associated cachexia. **Parathyroid hormone**-related protein (PTHrP) is the causative agent in humoral hypercalcemia of malignancy (HHM) and is frequently secreted from various kinds of solid tumors as well as from adult T-cell leukemia/lymphoma. PTHrP, like **PTH**, acts on **PTH** receptor type 1 (PTH1R). Activation of PTH1R may lead to stimulation of secretion of proinflammatory cytokines. It is expected, therefore, that PTHrP constitutes a key factor in the activation of the proinflammatory and cachectogenic cytokine network and consequently in the development of cachexia in patients with cancer. METHODS: Two groups of cancer-bearing patients of similar clinical backgrounds were enrolled. Plasma concentrations of PTHrP and cytokines were measured with immunoradiometric assay and radioimmunoassay, respectively. Cancer tissues from patients with HHM were transplanted into nude **mice** or nude **rats**. The effects of humanized anti-human PTHrP **antibody** were examined. RESULTS: In clinical studies, Group B patients (with elevated plasma PTHrP), compared with Group A patients (with normal plasma PTHrP), tended to exhibit higher plasma levels of tumor necrosis factor (TNF)-alpha ($P = 0.13$), interleukin (IL)-5 ($P = 0.08$), and IL-8 ($P = 0.08$), and had significantly higher levels of IL-6 ($P = < 0.05 = 0.01$). The levels of TNF-alpha and IL-6 correlated with those of PTHrP. In animal studies, the **antibody** caused a prompt and sustained decline in serum calcium. This response was accompanied by improvements in food intake, drinking, body weight gain, and general behavior. It also ameliorated the suppression of serum ADH. When those effects were compared with those induced either by bisphosphonate or calcitonin, it turned out that not all of the beneficial effects of the **antibody** were directly correlated with the depression of blood calcium. CONCLUSIONS: PTHrP is a promising molecular target for the development of a novel mode of treatment for patients with cancer-associated morbidity.

L18 ANSWER 6 OF 4 MEDLINE

DUPLICATE 2

2000461279 Document Number: 20237367. PubMed ID: 11241171. Nuclear

localization of the type 1 **PTH/PTHrP** receptor in **rat** tissues. Watson P H; Fraher L J; Heney G N; Chung V I; Kisiel M; Natale B V; Hodsman A B. (Department of Medicine, University of Western Ontario, and The Lawson Research Institute, London, Canada.) *JOURNAL OF BONE AND MINERAL RESEARCH*, 2000 Mar; 15 (3): 103-44. Journal code: 0884-5131. ISSN: 0884-5131. Pub. country: United States. Language: English.

AB The localization of **PTH/PTH**-related peptide (PTHrP) receptor (PTH1R) has traditionally been performed by autoradiography. Specific polyclonal **antibodies** to peptides unique to the PTHrP are now available, which allow a more precise localization of the receptor in cells and tissues. We optimized the IHC procedure for the **rat** PTHrP using 5-micron sections of paraffin-embedded **rat** kidney,

liver, small intestine, uterus, and ovary. Adjacent sections were analyzed for the presence of PTHR mRNA (by *in situ* hybridization) and PTHrP peptide. A typical pattern of staining for both receptor protein and mRNA was observed in kidney in cells lining the proximal tubules and collecting ducts. In uterus and gut, the receptor and its mRNA are present in smooth muscle layers (PTHrP target) and in glandular cuboidal cells and surface columnar epithelium. This suggests that **PTH**, or more likely PTHrP, plays a role in surface/secretory epithelia that is as yet undefined. In the ovary, PTHR was readily detectable in the thecal layer of large antral follicles and oocytes, and was present in the cytoplasm and/or nucleus of granulosa cells, regions that also contained receptor transcripts. PTHrP protein and mRNA were found in the liver in large hepatocytes radiating outward from central veins. Immunoreactive cells were also present around the periphery of the liver but not within two or three cell layers of the surface. Clear nuclear localization of the receptor protein was present in liver cells in addition to the expected cytoplasmic/peripheral staining. PTHR immunoreactivity was present in the nucleus of some cells in every tissue examined. RT-PCR confirmed the presence of PTHR transcripts in these same tissues. Examination of the hindlimbs of PTHR gene-ablated **mice** showed no reaction to this **antibody**, whereas hindlimbs from their wild-type littermates stained positively. The results emphasize that the PTHR is highly expressed in diverse tissues and, in addition, show that the receptor protein itself can be localized to the cell nucleus. Nuclear localization of the receptor suggests that there is a role for **PTH** and/or PTHrP in the regulation of nuclear events, either on the physical environment (nucleoskeleton) or directly on gene expression.

L18 ANSWER 7 OF 34 MEDLINE
2000179217 Document Number: 20179217. PubMed ID: 11709493. Nuclear localization of the type 1 **parathyroid hormone**/
parathyroid hormone-related peptide receptor in MC3T3-E1 cells: association with serum-induced cell proliferation. Watson P H; Fraher L J; Natale B V; Kisiel M; Hendy G N; Hodzman A B. (Department of Medicine, University of Western Ontario and The Lawson Research Institute, London, Canada.. pwatsonl@julian.uwo.ca) . BONE, (2000 Mar) 26 (3) 221-5. Journal code: 8504048. ISSN: 8756-3282. Pub. country: United States.

Language: English.
AB We have recently demonstrated that the receptor for **parathyroid hormone** (**PTH**) and **PTH**-related peptide (PTHrP), PTHR, can be localized to the nucleus of cells within the liver, kidney, uterus, gut, and ovary of the **rat**. We set out to determine the localization of the PTHR in cultured osteoblast-like cells. MC3T3-E1, ROS 17/2.8, UMR106, and SaOS-2 cells were cultured in alpha-modified eagle medium containing 15% fetal calf serum under standard conditions. Untreated cells were grown on glass coverslips to 75-95% confluence and fixed in 1% paraformaldehyde. For experiments designed to examine cells synchronized by serum starvation, cells were grown on glass coverslips, starved of serum for 46 h, and then fixed at 1-h intervals for a total of 26 h after the addition of serum to the medium. Parallel sets of cells were pulsed with [³H]thymidine to track the DNA duplication interval. The PTHR was localized by immunocytochemistry using a primary **antibody** raised against a portion of the N-terminal extracellular domain of the PTHR. The results presented herein indicate that the PTHR attains a nuclear localization in each cell line examined. In UMR106 cells, PTHR immunoreactivity was restricted to the nucleus. After cell synchronization, MC3T3-E1 cells double approximately 14 h after the addition of serum. Immunocytochemistry for the PTHR in these cells showed that the receptor staining is initially diffuse for the first 6 h, then becomes more perinuclear in distribution by 12-16 h. Nuclear localization of the receptor is achieved approximately 16-20 h after the addition of serum and remains there throughout the mitotic phase. Intense staining of mitotic and postmitotic cells was observed. No change in cell

proliferation kinetics was observed in MC3T3-E1 cells cultured in the presence of 25 nM **PTH**(1-34). These data suggest an important role for the PTHR in the nucleus of MC3T3-E1 cells at the time of DNA synthesis and mitosis.

L18 ANSWER 8 OF 24 MEDLINE
1999427872 Document Number: 99427872. PubMed ID: 10499526. A role for interleukin-6 in **parathyroid hormone**-induced bone resorption in vivo. Grey A; Mitnick M A; Masiukiewicz U; Sun B H; Rudikoff S; Jilka R L; Manolagas S C; Insogna K. (Section of Endocrinology, Yale University School of Medicine, New Haven, Connecticut 06520-8020, USA.) ENDOCRINOLOGY, (1999 Oct) 140 (10) 4683-90. Journal code: 0375040. ISSN: 0013-7227. Pub. country: United States. Language: English.

AB **Parathyroid hormone** (**PTH**) exerts its regulatory effects on calcium homeostasis in part by stimulating the release of calcium from the skeleton. **PTH** stimulate bone resorption indirectly, by inducing the production by stromal/osteoclastic cells of paracrine agents which recruit and activate the bone-resorbing cell, the osteoclast. The identity of the stromal cell/osteoblast-derived paracrine factor(s) responsible for mediating the effects of **PTH** on osteoclasts is uncertain. Recently, it has been demonstrated that the cytokine interleukin-6 (IL-6), which potently induces osteoclastogenesis, is produced by osteoblastic cells in response to **PTH**. Further, we have reported that circulating levels of IL-6 are elevated in patients with primary hyperparathyroidism, and correlate with biochemical markers of bone resorption. Thus, IL-6 may play a permissive role in **PTH**-induced bone resorption. In the current studies, we demonstrate that low-dose **PTH** infusion in rodents increased serum levels of IL-6, coincident with a rise in biochemical markers of bone resorption. In **mice**, both acute neutralization and chronic deficiency of IL-6 were associated with markedly lower levels of biochemical markers of bone resorption in response to **PTH** infusion than were observed in animals with normal IL-6 production. Acute neutralization of IL-6 did not affect **PTH**-induced changes in markers of bone formation. These findings demonstrate that **PTH** regulates systemic levels of IL-6 in experimental animals, that IL-6 is an important mediator of the bone-resorbing actions of **PTH** in vivo and suggest that IL-6 plays a role in coupling **PTH**-induced bone resorption and formation.

L18 ANSWER 9 OF 24 MEDLINE DUPLICATE 3
2000056169 Document Number: 10056169. PubMed ID: 10588616. **Parathyroid hormone**-related peptide stimulates DNA synthesis and insulin secretion in pancreatic islets. Villanueva-Penacarrillo M L; Canezas J; de Miguel F; Redondo A; Valin A; Valverde I; Esbrit P. (Department of Metabolism, Fundacion Jimenez Diaz, Madrid, Spain.) JOURNAL OF ENDOCRINOLOGY, (1999 Dec) 163 (3) 403-8. Journal code: 0375361. ISSN: 0022-0795. Pub. country: ENGLAND: United Kingdom. Language: English.

AB **Parathyroid hormone** (**PTH**)-related protein (**PTHrP**) is present in the pancreatic islet. Recent data in transgenic **mice** suggest that **PTHrP** might modulate islet mass and insulin secretion. In the present study, we assessed the effect of the N-terminal **PTH**-like region of **PTHrP** on DNA synthesis in isolated **rat** islets. **PTHrP** (1-34), between 1 pM and 10 nM, for 45 h stimulated [³H]thymidine incorporation into **rat** islets. This effect was maximally induced, about 2.5 fold over control, by 10 pM of this peptide, decreasing thereafter. In contrast, **PTHrP** (38-64) amide or **PTHrP** (107-139) were ineffective in increasing DNA synthesis in islets. Using reverse transcription followed by PCR, we confirmed that **rat** islets express **PTHrP** and the type I **PTH**/**PTHrP** receptor. Addition of a neutralizing anti-**PTHrP** **antibody** to the incubation medium of proliferating islets decreased islet DNA synthesis by 30%. The effect of a

submaximal dose (30 pM) of PTHrP (1-34) on DNA synthesis in **rat** islets was abolished by 25 nM bisindolylmaleimide I, a protein kinase C (PKC) inhibitor, but not by 25 microM adenosine 3',5'-cyclic monophosphate, Fp isomer, a protein kinase A inhibitor. Moreover, 100 nM phorbol-12-myristate-13-acetate for 48 h also increased DNA synthesis 2-fold over controls in islets. PTHrP (1-34), at 100 nM, in contrast to 50 microM forskolin or 10 mM NaF, failed to affect adenylate cyclase activity in islet membranes. PTHrP, at 30 pM, was also found to increase 2-fold insulin released into the islet-conditioned medium within 24-48 h. Our results suggest that PTHrP is a modulator of pancreatic islet growth and/or function by a PKC-mediated mechanism.

L18 ANSWER 10 OF 24 SCISEARCH COPYRIGHT 2002 ISI (R)

1998:295687 The Genuine Article (E) Number: SA429. Effect of antagonism of the **parathyroid hormone (PTH)**

-related protein receptor on decidualization in **rat** uterus.

Williams K D (Reprint); Major B J; Martin T J; Moseley J M; Leaver D D. UNIV MELBOURNE, DEPT PHARMACOL, PARKVILLE, VIC 3052, AUSTRALIA (Reprint); ST VINCENTS INST MED RES, FITZROY, VIC 3065, AUSTRALIA. JOURNAL OF REPRODUCTION AND FERTILITY (JAN 1998) Vol. 112, No. 1, pp. 59-67. Publisher: J REPROD FERTIL INC. 22 NEWMARKET RD, CAMBRIDGE, ENGLAND CB5 3DT. ISSN: 0022-4251. Pub. country: AUSTRALIA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB **Parathyroid hormone**-related protein (PTHrP) was detected at $32.3 \pm 3.9 \text{ pmol l}^{-1}$ in uterine luminal fluid from immature **rats** treated with oestradiol. As mRNA encoding PTHrP has previously been localized to implantation sites in pregnant **rats**, the role of luminal PTHrP during pregnancy was explored. Infusion of a **parathyroid hormone (PTH)** PTHrP receptor antagonist, (Asn(10),Leu(11)-)PTHrP(7-34) amide, into the uterine lumen during pregnancy in **rats** resulted in excessive decidualization. This effect was also observed after intrauterine infusion of a monoclonal **antibody** raised against PTHrP. The effect of infusion of **PTH/PTHrP** receptor antagonist was dependent upon successful implantation, was dose-dependent and confined to the treated hem. A decrease in the number of apoptotic decidual cells in antagonist-infused uterine hem compared with vehicle or non-infused hem was detected immunohistochemically at day 13 of pregnancy, and this decrease is likely to contribute to the 'over-decidualization' observed. In pseudopregnant **rats**, infusion of **PTH/PTHrP** receptor antagonist into the uterine lumen resulted in an increase in uterine wet weight of the infused hem compared with the non-infused hem, indicating a direct effect on deciduoma formation. Thus, activation of the **PTH/PTHrP** receptor by locally produced PTHrP appears to be crucial for normal decidualization during pregnancy in **rats**.

L18 ANSWER 11 OF 24 MEDLINE

96281910 Document Number: 96281910. PubMed ID: 8676079. The role of gp130-mediated signals in osteoblast development: regulation of interleukin 11 production by osteoblasts and distribution of its receptor in bone marrow cultures. Komis R; Nagasawa H; Zhou H; Tamura T; Saito M; Taga T; Hilton D J; Suda T; Ng K W; Martin T J. St. Vincent's Institute of Medical Research, University of Melbourne, Victoria, Australia. JOURNAL OF EXPERIMENTAL MEDICINE, 1996 Jun 15; 185 (6, 1581-91. Journal code: 2981108. ISSN: 0022-1067. Pub. country: United States. Language: English.

AB Interleukin (IL)-11 is a multifunctional cytokine whose role in osteoblast development has not been fully elucidated. We examined IL-11 production by primary osteoblasts and the effects of **rat** monoclonal anti-mouse glycoprotein 130 (gp130) **antibody** on osteoblast formation, using a coculture of mouse osteoblasts and bone marrow cells. IL-1, TNF alpha, PGE2, **parathyroid hormone (PTH)** and 1 alpha,25-dihydroxyvitamin D3 (1 alpha,25(OH)2D3) similarly induced

production of IL-11 by osteoblasts, but IL-6, IL-4, and TGF beta did not. Primary osteoblasts constitutively expressed mRNAs for both IL-11 receptor (IL-11R alpha) and gp130. Osteotropic factors did not modulate IL-11R alpha mRNA at 24 h, but steady-state gp130 mRNA expression in osteoblasts was upregulated by 1 alpha,25(OH)2D3, **PTH**, or IL-1. In cocultures, the formation of multinucleated osteoclast-like cells (OCLs) in response to IL-11, or IL-6 together with its soluble IL-6 receptor was dose-dependently inhibited by **rat** monoclonal anti-mouse gp130 **antibody**. Furthermore, adding anti-gp130 **antibody** abolished OCL formation induced by IL-1, and partially inhibited OCL formation induced by PGE2, **PTH**, or 1 alpha,25(OH)2D3. During osteoclast formation in marrow cultures, a sequential relationship existed between the expression of calcitonin receptor mRNA and IL-11R alpha mRNA. Osteoklasts as well as OCLs expressed transcripts for IL-11R alpha, as indicated by RT-PCR analysis and *in situ* hybridization. These results suggest a central role of gp130 coupled cytokines, especially IL-11, in osteoclast development. Since osteoblasts and mature osteoclasts expressed IL-11R alpha mRNA, both bone-forming and bone-resorbing cells are potential targets of IL-11.

L18 ANSWER 12 OF 24 MEDLINE
95354590 Document Number: 95354590. PubMed ID: 7628377. Endotoxin induces **parathyroid hormone**-related protein gene expression in splenic stromal and smooth muscle cells, not in splenic lymphocytes. Funk J L; Lausier J; Moser A H; Shigenaga J K; Huling S; Nissenbaum R A; Strewler G J; Grunfeld C; Feingold K R. (Department of Medicine, University of California, San Francisco 94121, USA.) ENDOCRINOLOGY, (1995 Aug) 136 (8) 3412-21. Journal code: 0013-7227. Pub. country: United States. Language: English.

AB **PTH**-related protein (PTHrP), the peptide that is responsible for most cases of hypercalcemia of malignancy, is also produced under normal circumstances by a variety of tissues. Its role and regulation at these sites are not well understood. Recently, we have shown that PTHrP is induced in the spleen during the host response to endotoxin (LPS) and that tumor necrosis factor (TNF) is a major mediator of this effect. Given the large body of *in vitro* evidence suggesting that PTHrP can be produced by lymphocytes and act in an autocrine loop to alter their function, studies were undertaken to determine whether lymphocytes were the cells responsible for PTHrP production in the spleen. Both constitutive and LPS-induced PTHrP messenger RNA (mRNA) levels were the same in **mice** lacking mature T cells (nude **mice**) and in **mice** lacking natural killer (NK) cells (due to pretreatment with **antibody** against NK 1.1) compared to levels in normal **mice**, suggesting that neither mature T cells nor NK cells were the splenic source of PTHrP. Even *sic* **mice** that lack functioning T and B cells responded to TNF with the induction of splenic PTHrP mRNA levels comparable to those in control **mice**. Localization of PTHrP mRNA in subfractions of **rat** spleens after *in vivo* treatment with LPS confirmed the results of the murine studies; PTHrP mRNA was barely detectable in the lymphocyte-rich single cell fraction of the spleen. In contrast, the stromal fraction of the spleen was enriched with PTHrP mRNA both in the basal state and in response to LPS. A similar pattern of distribution was seen for interleukin-6; LPS only increased mRNA levels of this TNF-inducible cytokine in the splenic stroma. In addition, mRNA for the **PTH/PTHrP** receptor, which decreased in response to LPS, colocalized with PTHrP mRNA in the stromal fraction of the spleen. Immunohistochemical studies identified PTHrP in two populations of splenic cells: 1) smooth muscle cells located in the splenic capsule and trabeculae and 2) a subpopulation of stromal cells located in the red pulp of the spleen, primarily in a subcapsular distribution. Consistent with the localization of PTHrP mRNA, lymphocytes in the white pulp of the spleen did not stain for PTHrP.

L18 ANSWER 13 OF 24 MEDLINE

96012243 Document Number: 96012243. PubMed ID: 7572312. Cell-to-cell communication in osteoblastic networks: cell line-dependent hormonal regulation of gap junction function. Donahue H J; McLeod K J; Rubin C T; Andersen J; Grine E A; Hertzberg E L; Brink P R. (Department of Orthopedics, Pennsylvania State University College of Medicine, Hershey, USA.) JOURNAL OF BONE AND MINERAL RESEARCH, (1995 Jun) 10 (6) 881-9. Journal code: 8610640. ISSN: 0884-0431. Pub. country: United States.

Language: English.

AB We have characterized the distribution, expression, and hormonal regulation of gap junctions in primary cultures of **rat** osteoblast-like cells (ROBs), and three osteosarcoma cell lines, ROS 17/2.8, UMR-106, and SAOS-2, and a continuous osteoblastic cell line, MC3T3-El. All cell lines we examined were functionally coupled. ROS 17/2.8 were the most strongly coupled, while ROB and MC3T3-El were moderately coupled and UMR 106 and SAOS-2 were weakly coupled. Exposure to **parathyroid hormone (PTH)** for 1 h increased functional coupling in ROB cells in a concentration-dependent manner. Furthermore, **PTH(3-84)**, an analog of **PTH** with binds to the **PTH** receptor and thus attenuates **PTH**-stimulated cAMP accumulation, also attenuated **PTH**-stimulated functional coupling in ROB. This suggests that **PTH** increases functional coupling partly through a cAMP-dependent mechanism. A 1 h exposure to **PTH** did not affect coupling in ROS 17/2.8, UMR-106, MC3T3-El, or SAOS-2. To examine whether connexin43 (Cx43), a specific gap junction protein, is present in functionally coupled osteoblastic cells, we characterized Cx43 distribution and expression. Indirect immunofluorescence with **antibodies** to Cx43 revealed that ROS 17/2.8, ROB, and to a lesser extent MC3T3-El and UMR-106, expressed Cx43 immunoreactivity. SAOS-2 showed little if any Cx43 immunoreactivity. Cx43 mRNA and Cx43 protein were detected by Northern blot analysis and immunoblot analysis, respectively, in all cell lines examined, including SAOS-2. Our findings suggest that acute exposure to **PTH** regulates gap junction coupling, in a cell-line dependent manner, in osteoblastic cells. (ABSTRACT TRUNCATED AT 250 WORDS)

L18 ANSWER 14 OF 24 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
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1995:505490 Document No.: PFEV199598510540. Osteoblastic cell lines derived from a transgenic mouse containing the osteocalcin promoter driving SV40 T-antigen. Chen, Di; Chen, Hong; Feng, Jian Q.; Windle, Jolene J.; Kop, Barbara A.; Harris, Marie A.; Eckenwald, Lynda F.; Breyre, Brendan F.; Worney, John M.; Mundy, Gregory R.; Harris, Stephen E. (1). (1) Univ. Texas Health Sci. Cent. at San Antonio, Dep. Med./Endocrinol., 7703 Floyd Curl Dr., San Antonio, TX 78284-7377 USA. Molecular and Cellular Differentiation, (1995) Vol. 3, No. 3, pp. 193-212. ISSN: 1065-3074. Language: English.

AB The object of this study was to develop new murine osteoblast-like cell lines for studying bone cell differentiation. In an attempt to develop cell lines representing a specific stage in osteoblast differentiation, we utilized transgenic **mice**. Immortalized cell-like cells were isolated and cloned from the calvaria of a transgenic mouse containing a 1.6-kb fragment of the **rat** osteocalcin promoter driving the expression of SV40 large T antigen. Two clonal cell lines, OCT-1 and OCT-2, were characterized. T-antigen expression by these two cell lines was confirmed using T-antigen **antibody**. Cell lines were tested for their responsiveness to **parathyroid hormone (PTH)**, prostaglandin E-2 (PGE-2), 1,25-dihydroxyvitamin D₃, (1,25D-3), bone morphogenetic protein-2 (BMP-2), transforming growth factor beta (TGF- β -eta), and retinoic acid. Their capacity to produce mineralized bone nodules and to express type I collagen, alkaline phosphatase (ALP), and osteocalcin at the mRNA level was also evaluated. Osteocalcin expression was found to be very low. OCT-1 and OCT-2 cells

injected into nude **mice** subcutaneously over the surface of calvaria caused osteosarcomas in 10 and 6 weeks, respectively. Significant new bone formation was associated with the tumors. OCT-1 and OCT-2 cells have different response profiles to BMP-2, retinoic acid, **PTH**, and PGE-2. These results demonstrate that OCT-1 and OCT-2 are cells representative of different stages of osteoblast differentiation. They have low levels of osteocalcin expression and may offer a tool to study the role of osteocalcin in bone formation and mineralization.

L18 ANSWER 15 OF 24 SCISEARCH COPYRIGHT 2002 ISI (R)
94:252375 The Genuine Article (R) Number: NG467. INACTIVATION BY PLASMA MAY BE RESPONSIBLE FOR LACK OF EFFICACY OF **PARATHYROID**-
HORMONE ANTAGONISTS IN HYPERCALCEMIA OF MALIGNANCY. KUKREJA S C
(Reprint); DANIA J J; WIMBISCUS S A; FISHER J E; MCKEE R L; CAULFIELD M P;
POSENBLATT M. W SIDE VET AIN MED CTR, ENDOCRINOL REPT MBL115, 920 N DAMEN
AVE, CHICAGO, IL, 60612 (Reprint); MERRICK HARP & JOHNS LTD, RES LABS, 11
POINT, PA, 19486; NICHOLS INST, SAN JUAN CAPISTRANO, CA, 92690; BETH
ISRAEL HOSP, BOSTON, MA, 02115. ENDOCRINOLOGY (MAY 1994) Vol. 134, No. 5,
pp. 2184-2188. ISSN: 0013-7227. Pub. country: USA. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB **PTH**-related protein (PTHrP) has been shown to be a major factor responsible for hypercalcemia of malignancy. PTHrP acts via the **PTH**/ PTHrP receptor, and therefore, **PTH** antagonists might be expected to reverse the hypercalcemia in malignancy. In the present studies, the **PTH** antagonists .cents.Tyr(14)-bovine (b)
PTH-(7-34)NH₂, .cents.D-Trp(12),Tyr(14)-f-**PTH**-(7-34)NH₂, or PTHrP-(7-34)NH₂, were administered to hypercalcemic athymic nude **mice** bearing a human squamous cell carcinoma of the lung in 50- to 500-fold molar excess of a dose of PTHrP-(1-34) known to produce hypercalcemia. The antagonists had no significant effect on serum calcium levels. In an adenylyl cyclase assay using the F93 17/2.6 cells, a potent **PTH** antagonist, .cents.Leu(11),D-Trp(12)-**PTHrP**-(7-34)NH₂ was rapidly inactivated in the presence of **rat** or human plasma. This inactivation by plasma was not blocked by common inhibitors of proteolysis (aprotinin, soybean trypsin inhibitor, and leupeptin). Preliminary studies demonstrated that inactivation of the PTHrP antagonist was caused by a plasma component with an apparent mol wt of 230,000 daltons. The knowledge of the structure of the **PTH**/PTHrP receptor combined with the identification of a hormone-inactivating plasma factor should facilitate the design of **PTH**-antagonists that are effective *in vivo*.

L18 ANSWER 16 OF 24 MEDLINE DUPLICATE 6
95225986 Document Number: 95225986. PubMed ID: 7710637. Purification of meprin from human kidney and its role in **parathyroid** **hormone** degradation. Yamaguchi T; Fushimi M; Sugimoto T; Kido H; Chihara K. (Department of Medicine, Kobe University School of Medicine, Japan.) BIOLOGICAL CHEMISTRY HOPPE-SEYLER, 1994 Dec; 375 (12): 811-4. Journal code: 8503054. ISSN: 0177-3543. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Meprin (EC 3.4.24.15) is known to occur in the kidneys of **mice** and **rats**, but has not previously been found in human kidney. Here we report the isolation of meprin from human kidney and show that it has a role in the degradation of **parathyroid** **hormone** PTH in that organ. The purified human meprin had properties almost identical to those of **rat** meprin including molecular size, substrate specificity and inhibitor sensitivity, and it also cross-reacted well with an **antibody** raised against **rat** meprin. Both the purified human meprin and the microvillar membranes of human kidney readily hydrolyzed human **parathyroid** **hormone** [hPTH-(1-34)] into several fragments, whose amino acid sequences corresponded well to each other. Thus, meprin appears to play a major role in the **PTH**-degrading activity in the microvillar membranes of human kidney. Our results indicate that meprin, which so far

has mainly been investigated in **mice** and **rats**, is found not only in these rodents, but also in the human kidney, and suggest that its physiological role in humans is to degrade **PTH** in the kidney.

L18 ANSWER 17 OF 24 MEDLINE
94150234 Document Number: 94150234. PubMed ID: 8107517. E-cadherins identified in osteoblastic cells: effects of **parathyroid hormone** and extracellular calcium on localization. Babich M; Foti L R. (Department of Biomedical Sciences, University of Illinois College of Medicine, Rockford 61107-1897.) LIFE SCIENCES, (1994) 54 (11) PL201-8. Journal code: 0375521. ISSN: 0024-3205. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The presence and regulation of cadherin localization in osteoblastic cells were examined. Monoclonal **antibody** (ECD-1 that interferes with E-cadherin function prevented cell adhesion in UMR 106-H5 **rat** osteosarcoma cells and non-tumorigenic mouse calvarial MC3T3 E1 cells, whereas CCL39 fibroblast adhesion was not affected. Immunofluorescent **antibodies** (ECCD-2 and polyclonal L-CAMP P1) revealed cadherins are localized along the osteoblastic cell-cell boundaries. Exposure of UMR 106-H5 cells to bovine **parathyroid hormone** (1-84) (PTH; 10 ng/ml x 1 hr) or low calcium medium (1.0-0.025 mM) produced cellular retraction accompanied by intense immunofluorescence for cadherins throughout cells with a corresponding loss of punctate localization at remaining cell-cell adhesion points. Western immunoblot analysis indicated 108 kd and 115 kd cadherins are present, with a smaller 104.5 kd band that became predominantly associated with the cytosolic fraction of cells treated with **parathyroid hormone** or lowered calcium. The results demonstrate E like cadherins are present in osteoblastic cells and implicate a regulatory role for **parathyroid hormone** and calcium in cadherin function and localization.

L18 ANSWER 18 OF 24 MEDLINE
94075432 Document Number: 94075432. PubMed ID: 8253564. Plasminogen-dependent activation of latent transforming growth factor beta (TGF beta) by growing cultures of osteoblast-like cells. Yee J A; Yan L; Dominguez J C; Allan E H; Martin T J. (Department of Biomedical Sciences, Creighton University, School of Medicine, Omaha, NE 68178.) JOURNAL OF CELLULAR PHYSIOLOGY, (1993 Dec) 157 (3) 528-34. Journal code: 0050222. ISSN: 0021-9541. Pub. country: United States. Language: English.

AB Osteoblasts secrete transforming growth factor beta (TGF beta) as a biologically inert, latent complex that must be dissociated before the growth factor can exert its effects. We have examined the production and proteolytic activation of latent TGF beta (LTGF beta) by clonal UMR 106-01 **rat** osteosarcoma cells and neonatal mouse calvarial (MC) osteoblast-like cells in vitro. Synthetic bPTH-(1-34) increased the activity of tissue-type (tPA) and urokinase-type (uPA) plasminogen activators (PA) in cell lysates (CL) of UMR 106-01 cells. The concentration of active TGF beta in serum-free CM from cultures treated with bPTH-(1-34) and plasminogen was significantly greater than in CM from untreated controls and cultures treated with either bPTH-(1-34) or plasminogen alone. This effect occurred at concentrations of **PTH** (1-34) that increased PA activity and was prevented by aprtinin, an inhibitor of plasmin activity. Treatment with bPTH-(1-34) had no effect on the concentration of TGF beta in acid-activated samples of CM. Functional consequences of proteolytically activated TGF beta was examined in primary cultures of neonatal MC osteoblast-like cells. Human platelet IGF beta 1 caused a dose-dependent increase in the migration of these cells in an in vitro wound healing assay. Cell migration was also stimulated in cultures treated with bPTH-(1-34) and plasminogen together. This effect was blocked by an anti-TGF beta 1 **antibody**. The results of these studies demonstrate that (1) LTGF beta secreted by osteoblasts in vitro is activated under conditions where the plasmin activity in the cultures is

increased, and (2) the TGF beta generated by plasmin-mediated proteolysis is biologically active. We suggest that the local concentration of TGF beta in bone may be controlled by the osteoblast-associated plasminogen activator/plasmin system. Furthermore, since several calcistropic factors influence osteoblast PA activity, this system may have an important role in mediating their anabolic and/or catabolic effects.

L18 ANSWER 19 OF 24 MEDLINE
93107186 Document Number: 93107186. PubMed ID: 8380175.

Parathyroid hormone-related peptide as an endogenous inducer of parietal endoderm differentiation. van de Stolpe A; Karperien M; Lowik C W; Juppner H; Segre G V; Akou-Samra A B; de Laat S W; Defize L H. (Hubrecht Laboratory, Netherlands Institute for Developmental Biology, Utrecht.) JOURNAL OF CELL BIOLOGY, (1993 Jan) 120 (1) 235-43. Journal code: 0021-9576. ISSN: 0021-9576. Pub. country: United States. Language: English.

AB **Parathyroid hormone** related peptide (PTHrP), first identified in tumors from patients with the syndrome of "Humoral Hypercalcemia of Malignancy," can replace **parathyroid hormone** (PTH) in activating the PTH-receptor in responsive cells. Although PTHrP expression is widespread in various adult and fetal tissues, its normal biological function is as yet unknown. We have examined the possible role of PTHrP and the PTH/PTHrP-receptor in early mouse embryonic development. Using F9 embryonal carcinoma (EC) cells and ES-5 embryonic stem (ES) cells as in vitro models, we demonstrate that during the differentiation of these cells towards primitive and parietal endoderm-like phenotypes, PTH/PTHrP-receptor mRNA is induced. This phenomenon is correlated with the appearance of functional adenylate cyclase coupled PTH/PTHrP-receptors. These receptors are the mouse homologues of the recently cloned **rat** bone and opossum kidney PTH/PTHrP-receptors. Addition of exogenous PTH or PTHrP to RA-treated EC or ES cells is an efficient replacement for dBcAMP in inducing full parietal endoderm differentiation. Endogenous PTHrP is detectable at very low levels in undifferentiated EC and ES cells, and is up-regulated in their primitive and parietal endoderm-like derivatives as assessed by immunofluorescence. Using confocal laser scanning microscopy on preimplantation mouse embryos, PTHrP is detected from the late morula stage onwards in developing trophectoderm cells, but not in inner cell mass cells. In blastocyst stages PTHrP is in addition found in the first endoderm derivatives of the inner cell mass. Together these results indicate that the PTH/PTHrP-receptor signalling system serves as a paracrine or autocrine mechanism for parietal endoderm differentiation in the early mouse embryo, thus constituting the earliest hormone receptor system involved in embryogenesis defined to date.

L18 ANSWER 20 OF 24 SCISEARCH COPYRIGHT 2002 ISI (R)
92:181084 The Genuine Article (R) Number: HH513. 17-BETA-ESTRADIOL INHIBITS INTERLEUKIN-6 PRODUCTION BY BONE MARROW-DERIVED STROMAL CELLS AND OSTEOBLASTS IN VITRO - A POTENTIAL MECHANISM FOR THE ANTI-OSTEOPROTRIC EFFECT OF ESTROGENS. CIRIGIOLI G; VILKA P L; FAISERI G; BOSWELL S; BODER G; WILLIAMS D C; MANCAGNA S C. Reprint. RICHARD L ROUDEMEYER VET ADM MED CTR, ENDOCRINOLOGY & METAB SECT, 1451 W 10TH ST, INDIANAPOLIS, IN, 46202; INDIANA UNIV, DEPT MED & BIOCHEM, INDIANAPOLIS, IN, 46202; LILLY RES LABS, INDIANAPOLIS, IN, 46202. JOURNAL OF CLINICAL INVESTIGATION (MAR 1992) Vol. 90, No. 3, pp. 883-891. ISSN: 0021-9736. Pub. country: USA. Language: ENGLISH.

AB *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*
The effect of 17-keto-estradiol on interleukin-6 (IL-6) synthesis was examined in murine bone marrow-derived stromal cell lines, normal human bone-derived cells, and nontransformed osteoblast cell lines from **mice** and **rats**. In all these cell types IL-6 production was stimulated as much as 10,000-fold in response to the combination of

recombinant interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-alpha). Addition of 17-beta-estradiol in the cultures exerted a dose-dependent inhibition of IL-1-, TNF-, and IL-1 + TNF-induced production of bioassayable IL-6. Testosterone and progesterone (but not 17-alpha-estradiol) also inhibited IL-6, but their effective concentrations were two orders of magnitude higher than 17-beta-estradiol. 17-beta-estradiol also decreased the levels of the IL-6 mRNA. In addition, estradiol inhibited both TNF-induced IL-6 production and osteoclast development in primary bone cell cultures derived from neonatal murine calvaria. The TNF-stimulated osteoclast development was also suppressed by a neutralizing monoclonal anti-IL-6 antibody. This in vitro evidence suggests, for the first time, a mechanistic paradigm by which estrogens might exert at least part of their antiresorptive influence on the skeleton.

L18 ANSWER 21 OF 24 MEDLINE DUPLICATE 7
92315026 Document Number: 92315026. PubMed ID: 1617498. Development of monoclonal antibodies to **parathyroid hormone** -induced resorptive factors from osteoblast-like cells. Perry H M 3rd; Gurbani S. (Geriatric Research, Education, and Clinical Center, VA Medical Center, St. Louis, Missouri 63125.) CALCIFIED TISSUE INTERNATIONAL, (1992 Mar) 50 (3) 237-44. Journal code: 7905481. ISSN: 0171-967X. Pub. country: United States. Language: English.

AB **Parathyroid hormone (PTH)** induces osteoblast-like cells to secrete factors capable of increasing cellular bone resorption; these factors are extremely labile. We have partially isolated them from supernatants of **PTH**-stimulated ROS 17.2 cells using affinity chromatography. Products eluted from the matrix, carboxymethyl cellulose covalently linked to Biotracer Blue F3GA, (CM-AB) were used as immunogens in **mice**. Serum from these **mice** blocked the effect of **PTH**-stimulated supernatants in a bioassay for bone resorption (the bone rudiment system). Then, hybridomas were produced from spleen cells of these **mice**. Screening of these hybridoma cultures revealed a consistent blocking effect of supernatants at a dilution of 1:100 in the bioassay. At higher dilution (1:1000), however, fewer culture supernatants blocked this effect in the bioassay. Mixed hybridoma cultures have been cloned and subcloned. Evaluation of the resulting hybridoma supernatants revealed that supernatants from at least three clones were necessary to neutralize the effect of the **PTH**-induced, osteoblast-produced resorption factors in organ culture. Similarly, using sucrose density gradient analysis, it is necessary to use three **antibodies** to bind all the 35 S-labeled protein from supernatants of **PTH**-stimulated ROS cultures (equivalent to the fraction used as immunogen in the **mice**). The effect of **PTH** in organ culture is blocked by this same group of hybridoma supernatants. The **antibodies** appear specific for osteoblast-like cell resorption factors, as they do not block the effect of **PTH** on cAMP accumulation in ROS cell cultures. On the other hand, they do block the effects of dibutyryl cAMP on resorption in organ culture. (ABSTRACT TRUNCATED AT 350 WORDS)

L18 ANSWER 22 OF 24 MEDLINE DUPLICATE 8
91193551 Document Number: 91193551. PubMed ID: 1611695. Production and characterisation of monoclonal antibodies to **parathyroid hormone** (1-34). Logue F C; Perry B; Biggart E M; Chapman R S; Beastall G H. (Institute of Biochemistry, Royal Infirmary, Glasgow, U.K.) JOURNAL OF IMMUNOLOGICAL METHODS, (1991 Mar 21) 157 (2) 159-66. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB Monoclonal **antibodies** to the biologically active N terminal region of **parathyroid hormone (PTH)** suitable for use in the measurement of circulating **PTH** concentrations have proved difficult to produce. In this study, no serum **PTH**

antibody titres could be detected in **mice** using synthetic human **PTH** (1-34) (free or coupled to albumin) or **PTH** (1-10) (coupled to keyhole limpet haemocyanin) as immunogen. A consistent response to **PTH** (1-34) peptide was obtained in DA **rats**. We have produced five monoclonal **antibodies** to **PTH** (1-34) derived from the fusion of DA **rat** spleen cells and the mouse myeloma line X63 Ag.8.653. Bulk production of the **antibodies** was achieved using congenitally athymic **mice** for ascites production. **Antibody** assessment studies revealed the **antibodies** to be sensitive to the oxidation state of the methionine residues in **PTH** (1-34). Two of the **antibodies**, 3B3 and 6E3, were shown to be of potential use in measuring circulating **PTH** (1-84) when used in combination with available **antibodies** to C terminal **PTH**. A third **antibody**, 4G3, which failed to recognise **PTH** (1-84) when used in combination with 3B3, formed the basis of a specific assay for **PTH** (1-34).

L18 ANSWER 23 OF 24 MEDLINE

90115818 Document Number: 90115818. PubMed ID: 2153281.

Parathyroid hormone-related peptide gene is expressed in the mammalian central nervous system. Weir E C; Brines M L; Ikeda K; Burtis W J; Broadus A E; Robbins R J. (Section of Comparative Medicine, Yale University School of Medicine, New Haven, CT 06510.) PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1990 Jan) 87 (1) 109-12. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB A **parathyroid hormone**-related peptide (PTHRP) has been identified in human tumors associated with the syndrome of humoral hypercalcemia of malignancy. While **parathyroid hormone** (**PTH**) gene expression appears to be limited to the parathyroid glands, PTHRP mRNA has been identified in a variety of normal tissues. To investigate the apparent expression of the PTHRP in the central nervous system, we examined extracts of whole **rat** brain for PTHRP bioactivity by measuring adenylate cyclase-stimulating activity (ACSA) in a **PTH**-sensitive assay. Extracts consistently contained ACSA and this activity was completely inhibited by a PTHRP antiserum but was unaffected by a **PTH** antiserum. ACSA was found in a number of anatomic subregions of **rat** brain, being greatest in the cortex and telencephalon. RNase protection analysis revealed PTHRP transcripts in total RNA prepared from whole **rat** brain and from the same anatomic subregions. By in situ hybridization histochemistry, we found that the highest levels of PTHRP gene expression occurred in neurons of the cerebral cortex, hippocampus, and cerebellar cortex. These studies demonstrate that both PTHRP mRNA and biological activity are present in a number of regions of **rat** brain. The widespread expression of this peptide by multiple types of neurons suggests that the PTHRP may play a general role in neuronal physiology.

L18 ANSWER 24 OF 24 MEDLINE

8605518 Document Number: 8605518. PubMed ID: 355751. Identification of a 160-kDa membrane component which is modulated by **parathyroid hormone**. Weinshank R L; Liken P A. EUROPEAN JOURNAL OF BIOCHEMISTRY, (1985 Nov 15) 153 (1) 179-85. Journal code: 0117600. ISSN: 0014-2946. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.

AB Monoclonal **antibodies** have been produced against primary bone cells obtained from the collagenase digestion of mouse cranial bone. **Antibodies** were selected on the basis of their immunoglobulin class and those which were identified as IgG were further screened for their ability to inhibit cAMP accumulation in response to sub maximal doses of the 1-34 amino-terminal peptide of bovine **parathyroid hormone**, bPTH(1-34). Nine hybridoma clones were subsequently

characterized as inhibitory with respect to **parathyroid hormone (PTH)** responses in intact mouse cranial bone and which also identified a variety of membrane components from detergent extracts of surface-labeled primary bone cells. Five of these **antibodies** immunoprecipitated a membrane component with Mr of 80 000 that appeared to be a major component of the extract susceptible to surface-labeling with ^{125}I . All nine monoclonal **antibodies** were shown to bind to a suspended-cell preparation of primary bone cells with 2-3 orders of magnitude greater binding than that of control **antibodies**. Using this assay, one clone, designated 3G12 IgG, was observed to exhibit desensitization effects at the binding level with a time course and dose dependency for **PTH** pre-incubation that was similar to the establishment of the refractory state in other systems. In addition, the desensitization effect occurred at 37 degrees C but not at 4 degrees C. This **antibody** was shown to bind saturably to both intact mouse cranial bone and primary bone cells with an apparent affinity constant (K_a) in the range of 10(?) M. Inhibition of bone cAMP accumulation in response to 2.5 nM $\text{bPTH}(1-34)$ was directly correlated to the binding of 3G12 IgG to intact mouse calvariae. A maximum inhibition of approximately 85% was observed. 3G12 IgG immunoprecipitated a single membrane component, Mr 150 000, from NP-40 detergent extracts of ^{125}I -labeled primary mouse bone cells. The molecular mass of this component was also 150 000 daltons when run on polyacrylamide gel slabs under non-reducing conditions. Control and **PTH**-pre-treated bone cells were surface-labeled, detergent-solubilized and immunoprecipitated with 3G12 IgG in order to investigate further the desensitization effect at the molecular level. Incubation of bone cells with 1 microgram/ml $\text{bPTH}(1-34)$ for 45 min at 37 degrees C caused an increased susceptibility to surface-labeling with ^{125}I that was approximately three-fold higher in specific activity than that of control cells. (ABSTRACT TRUNCATED AT 400 WORDS)

=> s 13 and pig
L19 49 L3 AND PIG

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L20 5 L19 AND MONOCLONAL

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L21 1 DUP REMOVE 120 (4 DUPLICATES REMOVED)

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L21 ANSWER 1 OF 1 MEDLINE DUPLICATE 1
94283265 Document Number: 94283265. PubMed ID: 8013360.

Parathyroid hormone-related protein in the cardiovascular system. Burton D W; Brandt D W; Neftos L J. (Department of Medicine, University of California-San Diego 92161. J ENDOCRINOLOGY, (1994 Jul) 135 (1):13-61. Journal code: 1375043. ISSN: 0801-7127. Pub. country: United States. Language: English.

AB **PTH**-related protein (PTHRP) is expressed in a stretch-responsive manner in several types of smooth muscle. We previously demonstrated the production of PTHrP in adult rat heart muscle. In this study, we demonstrate the production of PTHrP in the cardiovascular systems of several mammalian species, including human. We demonstrate PTHrP by immunohistochemistry, using a panel of murine **monoclonal antibodies** to PTHrP epitopes that span the entire length of the human PTHrP amino acid sequence, quantitate the concentration of PTHrP in the rat cardiovascular system by region-specific RIAs, and measure the relative levels of PTHrP messenger RNA (mRNA) by competitive polymerase chain reaction. Immunohistochemistry studies demonstrated the presence of PTHrP

in the cardiovascular systems of humans, rats, **pigs**, and rabbits. The most robust expression was found in atria, followed by the large vessels, then ventricles. No difference was seen between the left and right sides of the heart. Double staining procedures revealed that PTHrP and atrial natriuretic peptide were coexpressed in some cells. Using RIAs and polymerase chain reaction, we demonstrated that atria contained a higher concentration of PTHrP than ventricles and that the relative PTHrP concentrations correlated to its mRNA concentrations in these two tissues. The concentrations of PTHrP in the smooth muscle surrounding the aorta and vena cava were comparable to those in atria. However, in these large vessels, the higher PTHrP levels did not correspond to its mRNA levels. Whereas the immunoreactive concentrations of PTHrP were similar in the atria, aorta, and vena cava, the mRNA levels in the aorta and vena cava were 3-fold lower than those in the atria. Certain PTHrP epitopes appeared to be differentially expressed in specific cardiovascular tissues. A comparison of region specific assays showed that immunoreactivity measured by immunoassays to PTHrP-(38-64) and PTHrP (109-141) were 3- to 5-fold greater than that determined by an immunoassay to PTHrP-(1-34). Our observations demonstrate that the atria, aorta, and vena cava contain the greatest amounts of PTHrP in the cardiovascular system. The discrepancy between the concentrations of PTHrP and its mRNA present in the aorta and vena cava suggest that the two may be regulated differently in these tissues. The widespread distribution of PTHrP suggests an important function for the protein in the cardiovascular system, possibly functioning as the calcium counterpart for the atrial natriuretic-sodium regulatory axis.

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L22 2 L19 AND POLYCLONAL

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L23 1 DUP REMOVE L22 (1 DUPLICATE REMOVED)

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L23 ANSWER 1 OF 1 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 1
97331431 EMBASE Document No.: 1997331431. Studies of melatonin effects on
epithelia using the human embryonic kidney-293 (HEK-293) cell line. Chan
C.W.Y.; Song Y.; Ailenberg M.; Wheeler M.; Pang S.F.; Brown G.M.;
Silverman M.. Dr. M. Silverman, MRC Membrane Biology Group, Department of
Medicine, University of Toronto, Toronto, Ont. M5S 1A8, Canada.
Endocrinology 138/11 (4-12-4739) 1997.

Refs: 28.

ISSN: 0013-7227. CODEN: ENDOAQ. Puk. Country: United States. Language:
English. Summary Language: English.

AB The expression of melatonin receptors (MR) of the Mel1a subtype in
basolateral membrane of guinea **pig** kidney proximal tubule
suggests that melatonin plays a role in regulating epithelial function.
To investigate the cellular basis of melatonin action on epithelia, we
sought to establish an appropriate *in vitro* culture model. Epithelial cell
lines originating from kidneys of dog (MDCK), **pig** (LLC-PK1),
opossum (OK), and human embryo (HEK-293) were each tested for the
presence of MR using ^{125}I -iodomelatonin (^{125}I -MEL) as a radioligand.
The HEK-293 cell line exhibited the highest specific ^{125}I -MEL binding. By
intermediate filament characterization, the HEK-293 cells were determined
to be of epithelial origin. Binding of ^{125}I -MEL in HEK-293 cells
demonstrated saturability, reversibility, and high specificity with an
equilibrium dissociation constant ($K(d)$) value of 23.8 ± 0.5 pM and a
maximum number of binding sites ($B(\text{max.})$) value of 1.17 ± 0.11 fmol/mg
protein ($n = 5$), which are comparable with the reported $K(d)$ and $B(\text{max.})$
values in human kidney cortex. Coincubation with GTPyS (10 μM) and

pertussis toxin (100 ng/ml) provoked a marked decrease in binding affinity (K_d) was increased by a factor of 1.5-2.0), with no significant difference in B_{max} . Melatonin (1 μ M) decreased the forskolin (10 μ M) stimulated cAMP level by 50%. HEK-293 cells do not express dopamine D1A receptor. Following transient transfection of HEK-293 cells with human dopamine D1A receptor (hD1A-R), exposure of the cells to dopamine stimulated an increase in the level of cAMP. Similarly, transient transfection of HEK-293 cells with rat glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic peptide (GIP), and **PTH** type 1 receptors, each resulted in an hormone inducible increase in cAMP levels. Surprisingly, only the stimulatory effect of dopamine could be inhibited by exposure to melatonin. The inhibitory effect of melatonin on dopamine D1-induced increase in cAMP was completely inhibited by pertussis toxin (100 ng/ml, 18 h). Immunoblot and immunocytochemical studies were carried out using two **polyclonal antibodies** raised against the extra and cytoplasmic domains of Mel receptor. Immunoblot studies using **antibody** against the cytoplasmic domain of Mel(1a) receptor confirmed the presence of a peptide blockable 37 kDa band in HEK-293 cells. Indirect immunofluorescent studies with both **antibodies** revealed staining predominantly at the cell surface, but staining with the **antibody** directed against the cytoplasmic domain required prior cell permeabilization. By RT-PCR, HEK-293 cells express both Mel(1a) and Mel(1b) messenger RNAs, but the messenger RNA level for Mel(1b) is several orders of magnitude lower than for Mel(1a). We conclude that HEK-293 cells express MR predominantly of the Mel(1a) subtype. Our evidence suggests that one of the ways that melatonin exerts its biological function is through modulation of cellular dopaminergic responses.

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= s 124 and PTH
L25 19 L24 AND PTH

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L26 7 DUP REMOVE L25 (12 DUPLICATES REMOVED)

= s 126 and antibody
L27 1 L26 AND ANTIBODY

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L26 ANSWEF 1 OF 7 CAPLUS COPYRIGHT 2002 ACS
2002:616279 Document No. 137:168278 Peptide antigens for producing
antibodies having specificity to bioactive intact parathyroid hormone.
Zahradnik, Richard J.; Lavigne, Jeffrey R. (USA). U.S.
Pat. Appl. Publ. US 2002110871 A1 20020815, 11 pp. (English). CEDEN:
USKMC1. APPLICATION: US 2000-730174 20001208.

AB The authors disclose peptide antigens corresponding to amino acid residues 3-12, 1-12, 2-17 and 1-15 of parathyroid hormone (**PTH**) and antibodies having an affinity to these peptides. The antigens and antibodies are useful in detg. bioactive intact **PTH** levels in serum, plasma, and/or cell culture media. The antibodies possess a high degree of species cross-reactivity, but substantially mitigated cross-reactivity to non-whole **PTH** peptide fragments and little to no recognition of the first amino acid residue of **PTH**.

L26 ANSWEF 2 OF 7 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
2002:473607 Document No.: PFEV200200473607. Third generation assay for
PTH and bone histomorphometry in children on dialysis. Salusky,
Isidro B. (1); Kuizcn, Beatriz D.; Facincani, Inalda; **Lavigne**,

Jeffrey; Zahradnik, Richard J.; Jueppner, Harald; Goodman, William G.. (1) Pediatrics and Medicine, UCLA School of Medicine, Los Angeles, CA USA. Pediatric Research, (April, 2002) Vol. 51, No. 4 Part 2, pp. 432A. <http://www.pedresearch.org/>. print. Meeting Info.: Annual Meeting of the Pediatric Societies' Baltimore, MD, USA May 04-07, 2002 ISSN: 0031-3998. Language: English.

L26 ANSWER 3 OF 7 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC. 2002:321048 Document No.: PREV200200311048. Relationship between a third generation assay for **PTH** and bone formation in children on dialysis. Salusky, I. B. (1); Kuizon, B. D. (1); Facincani, I. (1); **Lavigne, J.**; **Zahradnik, R. J.**; Jueppner, H.; Goodman, W. G. (1). Peds and Med, UCLA Sch Med, Los Angeles, CA USA. Journal of the American Society of Nephrology, (September, 2001) Vol. 12, No. Program and Abstract Issue, pp. 172A. <http://www.jasn.org/>. print. Meeting Info.: ASN (American Society of Nephrology) / ISN (International Society of Nephrology) World Congress of Nephrology San Francisco, CA, USA October 10-17, 2001 ISSN: 1046-6673. Language: English.

L26 ANSWER 4 OF 7 MEDLINE DUPLICATE 2
95117567 Document Number: 95117567. PubMed ID: 7817810. Comparison of two parathyroid hormone assays for the rat: the new immunoradiometric and the older competitive binding assay. Jara A; Ecker J; **Lavigne J**; Felsenfeld A. (Department of Medicine, Wadsworth Veterans Administration Medical Center, Los Angeles, California.) JOURNAL OF BONE AND MINERAL RESEARCH, (1994 Oct) 9 (10) 1629-33. Journal code: 6610642. ISSN: 0884-0431. Pub. country: United States. Language: English.

AB A competitive binding assay for parathyroid hormone (**PTH** INS) has been used since 1986 to measure **PTH** in rats. During the past year an immunoradiometric assay for the measurement of **PTH** (**PTH** IRMA) in the rat was developed. The purpose of the present study was to compare results obtained with the **PTH** INS and IRMA and to provide a framework for comparison for investigators who have used the **PTH** INS in previous studies. A total of 99 rats were studied; 27 rats had normal renal function, and 72 rats had surgically induced renal failure. In the azotemic rats, the magnitude of hyperparathyroidism was varied by changing the calcium and phosphorus composition of the diet. The correlation between the two **PTH** assays in the 99 rats was $r = 0.98$, $p < 0.001$. For the 27 rats with normal renal function, the correlation even within the narrow range of normal **PTH** values was significant, $r = 0.71$, $p < 0.001$. In the 72 azotemic rats, in which the highest INS **PTH** value was approximately 17 times normal, the correlation between the two **PTH** assays was $r = 0.38$, $p < 0.001$. The **PTH** IRMA provides distinct advantages, such as extended standard range, shortened incubation time, increased sensitivity, and technical simplicity, but our results indicate that the **PTH** INS provided an accurate measurement of **PTH**. Furthermore, our results should provide investigators who have used the **PTH** INS in previous studies with a framework for comparison with studies in which the **PTH** IRMA was used.

L26 ANSWER 5 OF 7 MEDLINE DUPLICATE 3
95226362 Document Number: 95226362. PubMed ID: 7711510. Determination of biactive rat parathyroid hormone (**PTH**) concentrations in vivo and in vitro by a 2 site homologous immunoradiometric assay. Schultz V L; Garner F C; **Lavigne J R**; Taveras J U. (Dental Research Center, University of North Carolina, Chapel Hill 27799-7449.) BONE AND MINERAL, (1994 Nov) 27 (2) 121-32. Journal code: 6610642. ISSN: 0169-6009. Pub. country: Ireland. Language: English.

AB A new homologous 2-site assay for rat parathyroid hormone (IRMA), developed by Immutopics, Inc., has been evaluated and compared with a bone cell cAMP bioassay. Circulating **PTH** for adult rats assayed with this IRMA are in the range $10-15$ pg/ml, and of the same order of magnitude

as published values for biologically active **PTH**. The standard curve for the IRMA was linear over the range 3.4-240 pg/ml of rPTH 1-34, and serum samples diluted in parallel with the standard curve. The within-assay and between-assay coefficients of variation ranged from 5.2% (n = 18) to 7.6% (n = 24) and 8.3% (n = 16) to 26.4% (n = 10), respectively. Serum **PTH** values (mean +/- S.E.) for parathyroidectomized rats were 3.5 +/- 0.6 pg/ml (n = 18) versus 10.3 +/- 1.4 pg/ml (n = 16) for intact non-mated rats. Calcium injections suppressed circulating **PTH** by 50%. Lactating rats had serum **PTH** levels 5-fold higher and vitamin D deficient rats 60-fold higher than non-mated controls. **PTH** secreted from parathyroid cells in vitro was in the range 60-490 pg/ml as determined by the IRMA. These values represented 85.0 +/- 9.0% of the comparable kit assay values, indicating that the IRMA detects only bi-active **PTH**.

L26 ANSWER 6 OF 7 MEDLINE DUPLICATE 4
87274084 Document Number: 87274084. PubMed ID: 3609153. Highly sensitive two-site immunoradiometric assay of parathyrin, and its clinical utility in evaluating patients with hypercalcemia. Nussham S R; **Zahradnik R J; Lavigne J R**; Brennan G L; Nozawa-Ung K; Kim L Y; Keutmann H T; Wang C A; Potts J T Jr; Segre G V. CLINICAL CHEMISTRY, (1987 Aug) 33 (8) 1364-7. Journal code: 3421549. ISSN: 0009-9147. Pub. country: United States. Language: English.

AB We have developed a highly sensitive, two-site immunoradiometric assay (IPMA) for human parathyrin (**PTH**) that is specific for the intact, secreted, biologically active 84 amino-acid peptide. This assay has several technical advantages: it does not detect even high concentrations of inactive carboxyl-terminal fragments, results are available within 24 h, and the detection limit for intact hormone is low (1 ng/L). The assay readily measures concentrations of **PTH** in all healthy subjects and distinguishes these values from low or undetectable **PTH** values observed in clinical situations in which **PTH** secretion is expected to be suppressed. We found complete separation of results from 37 patients with surgically proven hyperparathyroidism and those from 23 patients with hypercalcemia associated with malignancy, the latter having **PTH** values at or below the lower limits of normal for this assay. The sensitivity, specificity, and rapid turnaround time of this two-site IRMA should advance the laboratory evaluation of patients with disorders of calcium metabolism.

L26 ANSWER 7 OF 7 SCISEARCH COPYRIGHT 2002 ISI (R)
86:366424 The Genuine Article (P) Number: 07419. A 2 SITE IRMA FOR INTACT N-TERMINAL SPECIFIC PARATHYROID-HORMONE INS-PTH). **ZAHRADN IK R (Reprint); BRENNAN G; UNG K N. NICHOLS INST DIAGNOST, SAN JUAN CAPISTRANO, CA, 92675. CLINICAL CHEMISTRY (1986) Vol. 32, No. 6, pp. 1154.** Pub. country: USA. Language: ENGLISH.

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